

**DYNAMICS OF MOSQUITO SPECIES (DIPTERA: CULICIDAE): IMPLICATIONS ON  
VECTOR MANAGEMENT AND MALARIA PREVALENCE UNDER GLOBALCLIMATE  
CHANGE**

by

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## CERTIFICATION

The undersigned certifies that he has read and hereby recommends for acceptance by the Faculty of  
Science a dissertation titled:

**Dynamics of Mosquito Species (Diptera: Culicidae): Implications on Vector Management and  
Malaria Prevalence Under Global Change**, in fulfilment of the requirements for the degree of  
Master of Sciences in Biological Sciences (Applied Entomology) of BIUST.

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Date: .....

## **DEDICATION**

This dissertation is dedicated to my family and friends who supported and encouraged me through the hurdles and all the challenges encountered.

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## ABSTRACT

Malaria is considered the main cause of morbidity and mortality in most countries of the sub-Saharan Africa. Though elimination of the disease is a priority in some African countries such as Botswana, there are bottlenecks that make it difficult to achieve such target. Factors associated with the environment and mosquito-vector capacity for disease transmission have contributions towards challenges affecting disease elimination. These are dynamics which have not been explored in the country involving the presence of water bodies, efficacy of the insecticides currently registered and used for vector control and the prevailing climate change on vector performance. First, it was showed that water bodies indeed generally attract mosquito densities with high significant ( $p < 0.001$ ) reduction of mosquitoes with distance away from water bodies. Mosquitoes were trapped with Center for Disease Control (CDC) miniature light traps with distance (embankment, 50m, 100m and 3.5km) from the water bodies (rivers). Secondly, the efficacy of registered and currently used insecticides on *Anopheles arabiensis* (as per World Health Organization standards) was investigated. Results showed pyrethroids were less efficacious though generally fast in action. Prominent pyrethroid resistance was observed in Okavango than Ngamiland and Bobirwa malaria endemic districts, indicating differential pesticide resistance in space. Finally, the thermal tolerance to activity of *An. arabiensis* adults (female and male) and the larvae were assessed. Results showed females had a wider range of critical thermal limits (CTLs) as compared to males with implications on possible future malaria transmission in climate change scenarios. Overall, Okavango showed the highest densities of mosquitoes with prominent pyrethroid resistance. Furthermore, mosquitoes from the district showed a wider range of CTLs than other districts. Therefore, a recommendation is made on indoor residual spraying to consider rotational use of insecticides which may reduce selection pressure on mosquitoes as a strategy of managing resistance on mosquito vector that rest indoors. This work recommends alternative control methods to the endemic districts as mitigating strategies towards resistance management. The country may utilize the current novel molecular and biotechnological approaches, biological methods and alternatives of botanical 'soft' pesticides which are environmental friendly in the control of vector mosquitoes.

**CHAPTER 1**  
**INTRODUCTION AND LITERATURE REVIEW**

## 1.0 Introduction

Malaria is a global disease responsible for about one million deaths annually worldwide (World Health Organization (WHO), 2008). In 2012, there were an estimated 207 million cases of malaria which accounted for approximately 627 000 deaths (WHO, 2013). An estimated 3.4 billion people continue to be at risk of malaria, mostly in Africa and South East Asia with about 80% of malaria cases occurring in Africa (WHO, 2013). According to WHO, (1990), malaria is recognized as one of the major health problems in Africa and it is established that 90% of all malaria deaths, occur in sub-Saharan Africa (WHO, 2013), where most of the deaths occur amongst children less than five years (Griffin *et al.* 2014). According to Maurel, (1994), more than 40% of the world's population living in over 100 countries is exposed to the high risk of malaria. Recently, it is estimated that 3.2 billion people in 97 countries are at a risk of being infected with malaria (WHO, 2016). Moreover, the burden is more apparent in Africa because of general poor socio-economic status of the populace and their inability to mitigate against the malaria burden.

There is a huge burden for health and the economic growth of countries where malaria is endemic (WHO, 2013). In 2013, a total expenditure on malaria prevention and treatment was about US\$ 2.7 billion, which is only half of the estimated US\$ 5.1 billion required to reach coverage of presently used control interventions. The malaria burden is therefore estimated to reduce annual economic growth by about 1.3% (Okorosobo *et al.*, 2011; WHO, 2014). A recent analysis of malaria economic burden in Ghana, Tanzania and Kenya, reported that the average treatment costs per case, were US\$ 11.99, US\$ 6.79 and US\$20.54 respectively (Sicuri *et al.*, 2013). Malaria imposes a lot of socio-economic costs in sub-Saharan Africa and impedes economic development through several channels including the quality of life, fertility, population growth, saving and investment, worker productivity, premature mortality and medical costs (Sachs and Malaney, 2002; Gelband *et al.*, 2004). Overall, the interaction between malaria and poverty surrounding malarious areas, has limited economic opportunities for most people especially in sub-Saharan African countries (Pattanayak, 2006).

It is however asserted that recently, malaria cases are declining in Africa (O'Meara *et al.*, 2010; WHO, 2016; Deribew *et al.*, 2017; though see Ferrão *et al.*, 2017). Despite the decline, there are some challenges that make malaria epidemic difficult to eliminate. These challenges include among others,

factors associated with vectors within their environment such as exposure to insecticides, climate change and breeding habitats that may proliferate vector populations. In Botswana, malaria mosquito vectors and the *Plasmodium* parasites have established locally leading to the cause of morbidity and mortality. Currently, Botswana is at 0.1- 1% cases per a 1000 population and ~32% of the population live in six malaria endemic districts (Simon *et al.*, 2013). This work looked into the environment-mosquito interaction for their contribution to the malaria dynamics. Specifically, I focused on (1) effect of natural water bodies on mosquito abundance dynamics, (2) assessing the insecticide resistance of *An. arabiensis* mosquitoes using currently recommended active ingredients, and (3) determining the thermal biology of the *An. arabiensis* mosquitoes to help explain their fate in the face of climate change.

## **1.1 LITERATURE REVIEW**

### **1.1.1 The Mosquito**

Mosquitoes are insects belonging to the family Culicidae of the order Diptera (two winged) with three body parts (head, thorax and the abdomen) and like other insects, their pair of wings enable them to fly (Goma, 1966; Harbach and Knight, 1980). They are slender and small, about 3-6mm and are different from other flies by a forward- projecting proboscis, many scales on the thorax, narrow abdomen, wing veins and fringe of scales along the posterior margin of the wings (Service, 2012). According to Maurel (1994), there are more than 3200 species of mosquitoes, which have been identified, and a few of them can incubate the *Plasmodium* parasite, the causal agent of malaria.

### **1.1.2 Mosquitoes of medical importance**

The most economically significant mosquitoes, which are transmitters of pathogens to animals and man, belong to genera *Anopheles*, *Culex*, *Mansonia* and *Aedes* (Macdonald, 1957; Goma, 1966; Kalita *et al.*, 2014). Mosquitoes belonging to these genera are vectors for pathogens of various diseases such as malaria (*Plasmodium*), filariasis (worm- *Wuchereria bancrofti*), yellow fever (virus), dengue (virus), chikungunya (virus), zika (virus), Japanese encephalitis (virus) and hemorrhagic fever (virus) (Dandalo, 2007). The genus *Anopheles* is one of about forty genera of mosquitoes and only one that can transmit malaria to humans (Harbach, 2004). It is therefore important to note that mosquitoes do not cause malaria but they are rather transmitters of the parasite that causes malaria. The female *Anopheles* mosquito is the one that is able to transmit the parasite to its host (Maurel 1994; Prakash *et*

*al.*, 2004). During blood feeding, the saliva with the parasite is transferred from the infected female to the host. It is therefore, the need for blood (to mature eggs) that leads to the transmission of the parasite.

### **1.1.3 Malaria mosquito vectors of Africa**

In Africa, the most common mosquitoes transmitting malaria parasite are members of the *An. gambiae* complex and the *An. funestus* group (Ebenezer *et al.*, 2014). There are eight reproductively isolated *An. gambiae* complex species that are almost indistinguishable morphologically which include *An. amharicus*, *An. arabiensis*, *An. gambiae*, *An. quadriannulatus*, *An. melas*, *An. merus*, *An. bwambae*, and *An. coluzzii* (Coetzee *et al.*, 2013). The *An. gambiae* complex members are found throughout tropical Africa in sub-Saharan Africa (WHO, 1989). The *An. funestus* group is composed of nine sibling species with *An. funestus* sensu stricto being the most anthropophilic and predominant in numbers and geographical distribution (Derua *et al.*, 2015). Most of the malaria infections in Botswana (98%) are caused by *P. falciparum* vectored by the female *An. arabiensis* (Ministry of Health (MoH), 2007; MoH, 2015). The distribution of *An. arabiensis* is concentrated in the lower rainfall zones (<1000 mm) represented by drier savannah areas and when occurring in deserts, it is associated with river systems (Coetzee *et al.*, 2000).

### **1.1.4 Life cycle of mosquitoes**

Mosquitoes are holometabolous insects, and have four distinct stages of development which are the egg, larvae, pupae and adult. The first three stages are aquatic and their development takes average ~5-14 days depending on the species and temperature while the last adult stage is terrestrial (Beck-Johnson *et al.*, 2013; Burkett-Cadena, 2013). Maurel (1994) states that mosquitoes breed in water or damp locations in an optimum breeding temperature range of 20-30°C and humidity levels  $\geq 60\%$ . In temperatures below 20°C, hatching of the eggs takes a longer time since cold temperatures act as a strong ecological constraint in their development and survival (Maurel, 1994; Thomas *et al.*, 2012) whilst in tropical areas (~27°C), eggs take between one to two days to hatch (Service, 2012).

The male and female mosquitoes of the same species have to mate before the female could lay the eggs. Sperms may be deposited once and stored in the spermatheca for future fertilization of eggs (Clemets and Potter, 1967; Klowden, 2006). The laid eggs may change colour from white to brownish

or blackish, deposited singly or in rafts depending on species (Mattingly, 1975; Day, 2016). In tropical areas ( $\sim 27^{\circ}\text{C}$ ), eggs take between one to two days to hatch while in cooler climates (below  $20^{\circ}\text{C}$ ) eggs may take between one to two weeks (Service, 2012). A female *Anopheles* mosquito needs proteins from a blood meal to develop its egg until it's fully gravid. Hence soon after mating, they shift their behavior from mate (using sex pheromones) to host finding (based on carbon dioxide cues) based on their chemical ecology. It takes about 2-4 days (or longer in cool temperature climates) for a female blood fed mosquito to be gravid and may have four to five ovipositions (per lifetime). Eggs are laid on moist surfaces in batches between 30 to 500 eggs (Becker, 2010). The proportion of eggs that will survive to hatching depends on several factors including but not limited to climate, predation and pathogens (Blaustein, 1999).

After two days when the eggs hatch onto a larvae, it would occur in aquatic sites feeding on algae and organic debris (Kivuyo *et al.*, 2014). Larvae breathe at the surface of water usually using a siphon (Burkett- Cadena, 2013). *Anopheles* larvae would normally position parallel to the water surface because of its dorsally located siphon where they breathe. The siphon for *Culex*, *Mansonia* and *Aedes* species are located on the posterior end therefore the larvae positioned at an angle with the water surface (Tyagi *et al.*, 2015). There are four instar larvae that molt three times before the pupal stage (Shililu *et al.*, 2003).

The pupa of mosquitoes is an active aquatic stage which breathes at the surface through a pair of small trumpet-like structures on the thorax. It does not feed and its development takes two to three days in warm ( $\sim 27^{\circ}\text{C}$ ) tropics and a week or more in cool ( $<20^{\circ}\text{C}$ ) temperate areas (Burkett- Cadena, 2013). It is prone to predation however is able to move quickly below the water surface when disturbed (Shililu *et al.*, 2003).

The adult stage has a distinctive proboscis projecting forward adapted for feeding purposes (Walker, 2009). The antennae in males is known as plumose while in females is pilose (has few short hairs) and in both sexes of *Anopheles*, the maxillary palps are long, but clubbed in males (Goma, 1966). The adult is active during the night or dense shade and spends the day resting in grasses (Suárez-Mutis *et al.*, 2009). However, some of the mosquitoes have evolved abnormal behaviors of feeding or active during the day, owing to failure to find host at night due to control strategies that mainly target nights (Thomsen *et al.*, 2016). Under favorable conditions, males normally live for 10

days or less while females can live for about six to eight weeks. Females have potential to lay eggs about every three days during their lifespan. Most mosquitoes may have an average lifespan of less than two months (Briegel and Kaiser 1973; Ndoen *et al.*, 2012). However, mosquito longevity is not static, but is a function of several biotic and abiotic factors *vis a vis* temperature, rearing conditions and availability of biotic antagonists e.g. pathogens and parasites (Marinho *et al.*, 2016).

### 1.1.5 The *Plasmodium* parasite and the life cycle

Malaria is caused by four different protozoan species of genus *Plasmodium* and is transmitted by Anopheline group of mosquitoes (Maurel, 1994). These are *P. falciparum*, *P. vivax*, *P. malariae* and *P. ovale* (Maurel, 1994; Becker *et al.*, 2010). However, there is a recent species in the South-east Asia (*P. knowlesi*) originally from monkeys (White, 2008). *Plasmodium* is a single-celled eukaryote in the phylum Apicomplexa (Fig. 1). The main species of medical importance is *P. falciparum* (Cavalier-Smith, 1993; Sharma *et al.*, 2014; WHO, 2014), which accounts for the majority of deaths worldwide. *Plasmodium vivax* is widespread and causes a relapsing form of malaria, but accounts for far fewer deaths compared to *P. falciparum* (Gilles *et al.*, 1993).

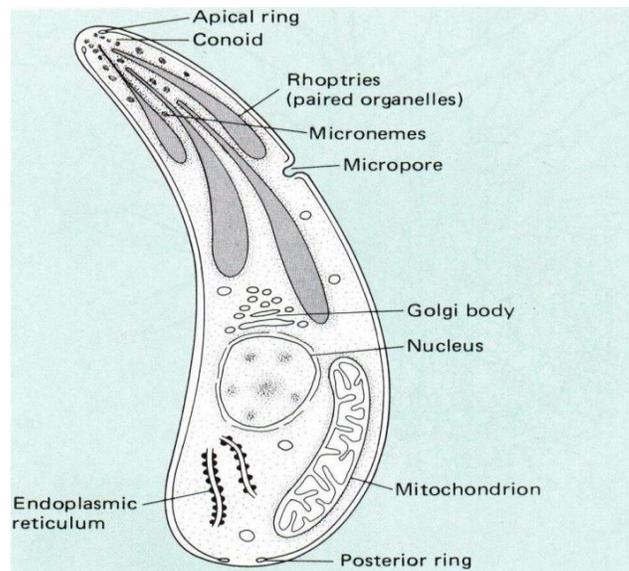


Figure 1: The structure of Apicomplexan protozoan, the pathogen for malaria. (Dorit, 1991).

The infection starts when sporozoites- bearing female *Anopheles* mosquito bites into the host's skin during a bloodmeal and the sporozoites (eggs) in the salivary gland are injected into the human's

bloodstream (Maurel, 1994). The parasite (eg *P. falciparum*) would then head for the liver cells where it would stay in an incubation phase for between 9-14 days (Brasil *et al.*, 2011). Maurel, (1994) documented that *P. ovale* and *P. vivax* may stay dormant in the liver for much longer. According to Dorit (1991), the sporozoites in the liver cells mature into schizonts (mother cells) which then produce numerous merozoites (daughter cells). These merozoites when released from the ruptured blood cells would invade new other blood cells in the bloodstream. The simultaneous release of the merozoites from the ruptured cells into the blood stream causes a fever (Maurel, 1994; Solomon *et al.*, 2014). The merozoites can also differentiate by meiosis to form male and female gametes into the bloodstream which the female *Anopheles* mosquito can ingest during a bloodmeal (Dorit, 1991). The asexual cycle lasts approximately 48 hours in *P. falciparum*, *P. vivax* and *P. ovale* infections and up to 72 hours in *P. malariae* infection (Garnham, 1988; Sun *et al.*, 2014).

When the female mosquito bites an infected human being, the gametocytes merge into a zygote (in the midgut) which would develop into ookinetes and the motile ookinetes then penetrate the midgut walls and develop into oocysts (Dorit, 1991). The cysts would eventually release sporozoites which then migrate into the salivary glands where they get injected into humans. For the life cycle to be completed, the *Plasmodium* has to undergo developmental phases in an Anopheline mosquito and in humans as host (Fig. 2).

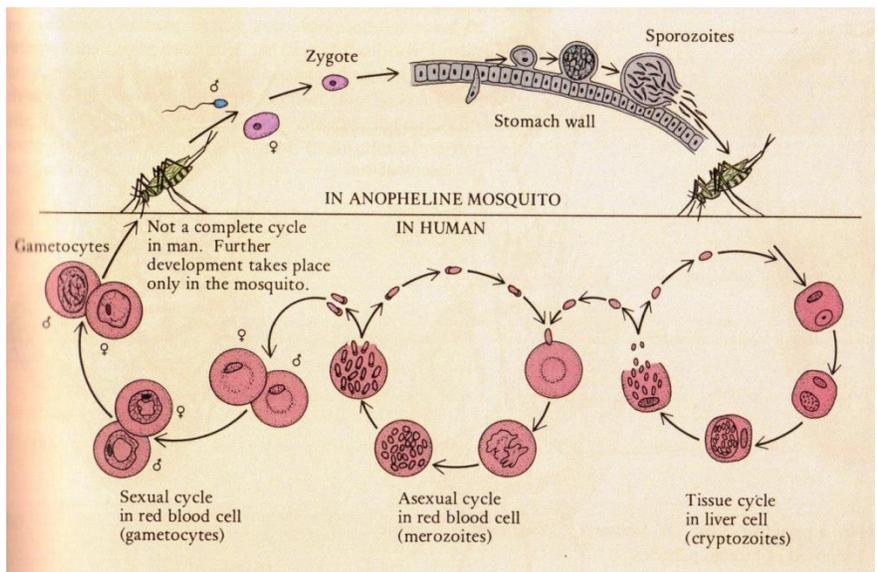


Figure 2: Life cycle of a Plasmodium parasite in Anopheline mosquito and in human hosts: Dorit (1991).

## **1.1.6 Ecology of mosquitoes**

### **1.1.6.1 Breeding habitats and Oviposition**

Female mosquitoes that are ready to lay their eggs need to select an oviposition site as a most important component of their survival (Bentley, 1989; Artis *et al.*, 2014). Water with decaying organic matter (as food for immature) is a strong pre-oviposition attractant to gravid female mosquitoes (Okal *et al.*, 2013; Huang *et al.*, 2006). Most mosquito species breed at water temperature of 26.5°C to 29.3°C and pH range of 7.1 to 7.3 (Afolabi *et al.*, 2013). A pH less than 5.0 and slightly higher than 7.4 can produce a lethal effect on mosquito species (Adebote *et al.*, 2006). Mosquitoes lay their eggs in aquatic habitats in different locations through a behavior called skip-oviposition to increase chances of survival (Colton *et al.*, 2003). Some Anopheline mosquito species prefer to lay eggs in habitats associated with vegetation compared to sites with bare soil, thus plants acting as chemical clues for oviposition (Overgaard, 2007). Habitats with conspecific larvae may attract gravid mosquitoes indicating suitability of the habitat for a species (Sumba *et al.*, 2004). In some species of Culicine mosquitoes, microbes and their metabolites act as oviposition attractants and stimulants (Trexler *et al.*, 2003). Competitors and predators found in aquatic habitats, may release kairomones which the female mosquitoes may detect, and thus avoid investing reproductively in those habitats due to their high offspring mortality (Siberbush and Blausten, 2008).

### **1.1.6.2 Feeding Behavior**

According to Chaves *et al.*, (2010), mosquitoes have several biting patterns. Some mosquitoes prefer to bite in the house (endophagic), while others bite mainly outdoors (exophagic). Mosquitoes which rest in the house after blood feeding are known as endophilic while exophilic refer to those that rest outdoors after a blood meal. Maurel (1994) suggests that *Anopheles* mosquitoes prefer biting indoors and at night, often about dawn and dusk. However, over the years Anopheline mosquitoes shifted from feeding inside to outside and from humans to animals (Garrett-Jones, 1980) and these evolutionary changes are due to them being targeted by indoor insecticides and other control strategies (Ferguson *et al.*, 2010). Endophilic species can feed outside to some degree and may increasingly do so in response to domestic interventions (Govella *et al.*, 2010). A study in Benin showed that the high prevalence of outdoor frequent biting by malaria vectors was due to the use of both impregnated nets and indoor residual spraying as compared with the use of impregnated nets alone (Corbel *et al.*, 2012).

In three years, *An. funestus* changed its host seeking and biting behavior from biting at night to early mornings and afternoons (Moiroux *et al.*, 2012).

### **1.1.6.3 Mosquito dispersal**

Mosquito dispersal is possible in three different ways including dispersal on human transportation and trade (Reiter, 1998), wind- assisted (passive) and short daily flights in search of hosts (Harrington *et. al.*, 2005), nectar, mates, oviposition and resting sites (Verdonschot and Besse-Lototskaya, 2013). According to Bell *et al.* (2005), survival rate during wind-assisted dispersal is very low due to changing temperatures and humidity over long distances. A study by Bogojevic *et al.* (2011) showed that the average mosquito dispersal rate per day ranged from 0.96km to 3.6km depending on species. Kaufmann and Briegel (2004) categorize mosquitoes into strong (> 2km) and weak (< 2km) flyers. Thus, *Culex* species can travel a minimum of 0.16km and a maximum of 1.98 km (Ciota *et al.*, 2012). In general, mosquitoes are not strong flying insects however as these factors (as above) favor them, they can invade new habitats across borders and into non disease endemic zones thus risking establishing their populations capable of diseases outbreaks.

### **1.1.6.4 Mosquito- microbial interaction**

Mosquitoes are known to have bacterial endosymbiosis that play roles in key insect function such as nutrition, reproduction, development and protection against enemies (Douglas, 2011). Bacteria can colonize different organs in mosquitoes, mainly the midgut and to a lesser extent, salivary glands and the reproductive organs (Zouache *et al.*, 2011). In *Anopheles* mosquito species, bacterial diversity is thought to be low at adult stage because of gut renewal during metamorphosis from pupae to adult (Pumpuni *et al.*, 1996). In southern Zambia, a study done by Cirimotich *et al.* (2011) showed that *Enterobacter* bacterium isolated from wild mosquito populations rendered the mosquito 99% resistant to *P. falciparum* by interfering with parasite development and invasion to the midgut epithelium and that a low density of only 100 ingested bacteria was able to decrease oocyst intensity by 67%. Indeed, if parasite does not develop, the transmission is also limited. Also that, the clearing of the gut microbiota in mosquitoes using an antibiotic, resulted in enhanced *Plasmodium* infections therefore giving conclusion that, microbiota can defend against *Plasmodium* parasite (Dong *et al.*, 2009).

### 1.1.7 Vector control

A number of techniques have been used to control and prevent malaria disease and its transmission (Shan-Qing *et al.*, 2014). There are two main strategies used worldwide in malaria control which are; treating the disease and vector control. According to Takken and Knols (2009), effective control practices include education of the public about malaria and the role of the mosquitoes in the transmission. Control strategies include house and environment modifications to reduce mosquito entry, larval development sites reduction and treatment, use of bednets, repellents and indoor residual spraying (IRS) of insecticides. Insecticides treated bednets (ITNs) programme has substantially reduced mortality and disease prevalence over the years (Lenger, 2004; Gunay *et al.*, 2014). The distribution of nets as a means of vector control, whether as donations or at low cost, is still an active area of economic burden (Cohen and Dupas, 2010). Pluess *et al.*, (2010) suggests that IRS results in death of mosquitoes as they rest in houses following blood meals and that the campaigns on IRS have shown significant effects on disease prevalence. However, malaria control remains a challenge in African communities including but not limited to lack of knowledge (illiteracy), ignorance and poverty. Botswana shares similar challenges also including housing structures (made of mud, reeds and grasses) that encourage not only mosquito entry into houses but also hiding and resting places (Fig. 3B) and the general misconception of people not preferring the use of ITNs (Fig. 3A).

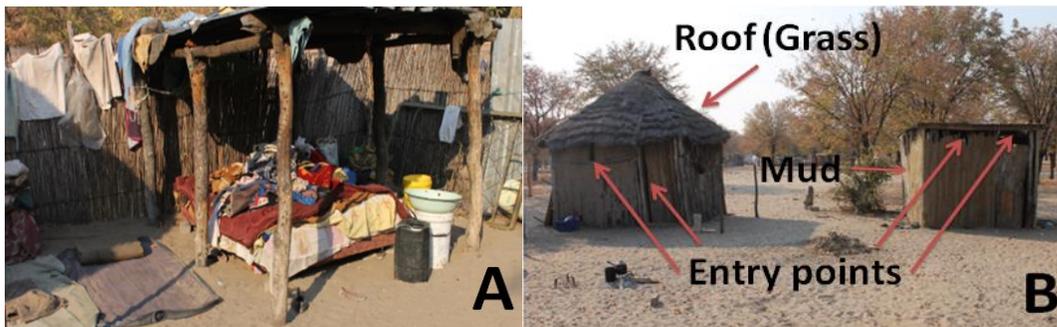


Figure 3: Typical traditional housing structures taken in northern Botswana (A) outdoor sleeping spaces without protection of insecticides treated bednets (ITNs) and (B) African traditional houses showing many mosquito entry points.

#### 1.1.7.1 Biological control

Biological control techniques have been widely used and appreciated in mosquito control, including the use of predators and parasitoids (Takken and Knols, 2009). Predators that feed on

mosquitoes are important biological control of disease vector (Becker *et al.*, 2014). A study done by Weterings *et al.* (2014) on salticids spiders demonstrated a valuable biological control for adult mosquitoes. The *Plexippus* species (of salticids spiders) has been able to consume high numbers of adult mosquitoes with an average of 6.0 ( $\pm 0.8$ ) mosquitoes per day for male spiders and 9.2 ( $\pm 0.85$ ) for female spiders (Weterings *et al.*, 2014). Recently, the population of *Anopheles* mosquitoes has been controlled sustainably by the introduction of the Dragonfly (Odonata) which feeds on all developmental stages (Faithpraise *et al.*, 2014). Parasitoids have also been used as a biological control method on mosquitoes. Parasitoids are organisms usually insects, part of whose developmental stage survives/ develops inside another host, by feeding in the body of other organisms such as insects (Godfray, 1994). This control method has been tried on mosquito larvae of *Ae. aegypti* infected with mermithid nematodes. The parasitized larvae exhibited an impaired feeding behavior and less active in its late developmental stage (Wise de Valdez, 2006). Recently, the use of an endosymbiotic bacterium, *Wolbachia*, is used as a biological control to shorten life- span in some mosquito species by interfering with pathogen replication and dissemination (McMeniman *et al.*, 2009).

#### **1.1.7.2 Genetic control**

Sterile insect technique (SIT) is a recently used method of genetic approach in insect control with the aim to reduce wild ‘problem’ insect population (see though novel biotechnological approaches; Joga *et al.*, 2016). It is a species-specific form of birth control involving the mass rearing of males, sterilized through chemicals or radiation and realized to mate with a wild population (Dyck, 2006). Sterile insect technique has been used as a pest management tool to control insect pests with success stories on fruit flies, melon flies, screwworm, tsetse flies and mosquitoes (Knipling, 1985; Vreysen *et al.*, 2000; Koyama *et al.*, 2004; Vargas-Terán *et al.*, 2005; Ant *et al.*, 2012; Bouyer and Lefrançois, 2014). In Brazil, a transgenic *Ae. aegypti* (*OX513A*) strain was released to suppress wild population and successfully reduced dengue disease transmitted by *Ae. aegypti* mosquitoes (Carvalho *et al.*, 2014). Indeed, efforts are now being made also to reduce malaria by applying SIT on vector species (Dame *et al.*, 2009; Klassen, 2009). Galiziet *et al.* (2014) showed a population suppression of wild *An. gambiae* populations by male mosquitoes. This was possible through male mosquito sex distortion by shredding the paternal X chromosome preventing it from being transmitted to the next generation. The technique resulted in fully fertile mosquito strains that produce >95% male offspring.

Sterile insect technique may also result in successful mating resulting in eggs that are not viable or offsprings that do not survive (Robinson, 2005).

### **1.1.7.3 Chemical control (Insecticides)**

Insecticides are chemical compounds that have been discovered naturally to have a negative effect on the life of insects and other arthropods (Oberemok *et al.*, 2015). The discovery was due to the agricultural losses caused by a variety of insect pests (Pimental, 1976; Walker, 1983; Oerke, 2006). Furthermore, some insects brought about a burden of disease to humans and animals (Steinhaus, 1957; Sarwar, 2015) therefore control measures had to be established by means of ‘chemicals’ (Friend, 1952; Simarro *et al.*, 2011). Over the years, mosquitoes have been successfully controlled by the use of chemicals (Konradsen *et al.*, 2004). However with time they developed resistance to the insecticides thus, currently chemical control of mosquitos remains a challenge (Cui, 2006; Coleman *et al.*, 2017).

The chemical control has been used in mosquitoes as an application for interference methods that are supported by the theoretical foundations of mosquito chemical ecology. These are the semiochemicals that carry information or chemical cues for a given species and the interactions thereof, thus triggering behavioral or physiological responses in receiving organisms (El-Shafie and Faleiro, 2017). For instance, the allelochemicals may favor the emitting species (allomones) or the receiving species (kairomones) whilst the synomones favor both species (Ilori and Ilori, 2012). Moreover, pheromones are substances secreted by species for others to receive thus, provoking specific behavioral responses such as sexual, aggregation, dispersion, alarm, territoriality, trail, oviposition and others (Mordue 2003). Overall, these chemicals have been ‘mimicked’ and used in products that repel, attract and kill mosquitoes for their control (eg. Effiom *et al.*, 2012).

#### **1.1.7.3.1 Classes of insecticides**

There are different types of pesticides in the market capable of eliciting resistance in insects broadly categorized as organic and inorganic (Bahlai *et al.*, 2010). Organic insecticides are based on chemicals having carbon as the basis of their molecular structure. The chemicals in organic pesticides are more complex than those of inorganic insecticides and usually do not dissolve easily in water. Inorganic pesticides are simpler compounds. They have a crystalline, salt-like appearance, are environmentally stable and usually dissolve readily in water. Inorganic insecticides are those that do not contain carbon and are usually of mineral origin, mainly compounds of antimony, boron, copper,

fluorine, mercury, selenium, sulfur, thallium and zinc, and elemental phosphorus and sulfur (Sarwar, 2016). There are four most common organic synthetic pesticides which are the chlorinated hydrocarbons, organophosphates, carbamates and pyrethroids (Zhu *et al.*, 2014). In Botswana, lambda-cyhalothrin (pyrethroid) and Dichloro Diphenyl Trichloroethane (DDT; organochlorine) are the main insecticides used in mud and/or unplastered walls and painted walls respectively. For purposes of vector control in the country, WHO has recommended organophosphates (malathion, fenitrothion and pirimiphos methyl), organochlorines (DDT and dieldrin), carbamates (propoxur, bendiocarb and carbosulfan) and pyrethroids (lambda-cyhalothrin, permethrine, deltamethrin, cyfluthrin and etofenprox) also, the pyrroles (chlorfenapyr) and phenyl pyrazoles (fipronil). Therefore, in establishing a baseline study towards resistance in mosquitoes, there was a need to assess Anopheline mosquito susceptibility to the above mentioned currently registered insecticides.

#### **1.1.7.3.2 Insecticides mode of action**

Insecticides can target the insect cuticle formation, hormonal and nervous system, energy production, enzyme inhibition, electron transfer inhibition, water and pH balance (Casida, 1956; Das, 2013; Swale *et al.*, 2014; Mpumi *et al.*, 2016). To be acceptable, pesticides must not have strong toxicity toward non-target organisms and the environment. Yet, to be efficient, they must be highly toxic toward their intended targets. The mechanism of this type of selectivity is often the targeting of a molecular target site that is found only in the pest or, if in other organisms, is particularly vulnerable in the pest; e.g., an enzyme form that is significantly different from that of other organisms. Overall, insecticides are site specific in their mode of action. Carbamates and the organophosphates insecticides inhibit the enzyme acetyl cholinesterase in insects while the pyrethroids and the organochlorines target the sodium ion channels (Tiwari *et al.*, 2012; Zhang *et al.*, 2016)

#### **1.1.7.3.3 Insecticide resistance**

Insecticide resistance may be defined as a “genetically based decrease in susceptibility to a pesticide” (Tabashnik *et al.*, 2014). However, Dekker (1986) defines it as the inherited ability in a strain of a pest to tolerate doses of a toxicant that would otherwise prove lethal to a majority of individuals in a normal population. Evolution of pest resistance to pesticides is an increasingly urgent problem that threatens human health and agriculture worldwide (Brent and Holloman, 2007; Coetzee and Koekemoer, 2013).

The first documentation on insecticide resistance was published 100 years ago and involved lime sulfur and the San Jose scale (Melander, 1914). Thereafter, a few sporadic cases of insecticide resistance were reported through the mid-1940s (Forgash, 1984). Whalon *et al.* (2013) has recorded resistance in at least 546 species of arthropod insect pests, 218 species of weeds, and 190 species of plant pathogens. Insects develop resistance due to the insecticides used upon them as a control measure. As such, pesticide pressure enhances resistance within the population through natural selection (Raymond and Marquine, 1994). Furthermore, the establishment and advancement of insecticide resistance depends on effective gene flow within the population (Barnes *et al.*, 2017). Also the environment associated factors (such as temperature and humidity) play an important role in resistance mediation to insect population (Oliver and Brooke, 2017). An insect pest species can be resistant to one pesticide in a certain geographic location and a particular timing (Mota-Sanchez *et al.*, 2006) while some can involve even more than one pesticide (Hemingway *et al.*, 2002). In malaria mosquito vectors, insecticide resistance is widely reported (including Africa) especially against the pyrethroids (Chaumeau *et al.*, 2017; Chouaibou *et al.*, 2017). In Botswana pyrethroids and DDT (with same target site) have been used over the years. However, it remains unclear whether these pesticides remain efficacious against target mosquitos. In consequence, resistance testing and mechanisms thereof and monitoring of its development is highly warranted.

#### **1.1.7.3.4 Mechanisms of resistance**

While pesticides are designed to target certain sites, insects have evolved mechanisms of resistance against them such as metabolic, target site, penetration, behavioral, cross and multiple resistances (Liu, 2015). Metabolic resistance is basically the biochemical transformation of a toxin, wherein the toxic compound is transformed into a less toxic form (Li and Schuler, 2007). The detoxification of synthetic chemicals by insects is mainly associated with cytochrome P450s and with large multigene families such as esterases, oxidases, and transferases (Terriere, 1984; Scott, 1999). Amongst these detoxifying enzyme systems, cytochrome P450s are involved in the metabolism of pyrethroids; Glutathione S-transferases (GSTs) detoxify organochlorine compounds; and esterases and oxidases act primarily on carbamates (Feyereisen, 1999).

Insecticides react with certain target sites (Acetylcholinesterase, gamma aminobutyric acid-gated chloride channels, etc.) in the organism thereby inhibiting their enzymatic activity. However, some blood-feeding insects are thought to have evolved resistance to organophosphate and carbamate

(Komagata *et al.*, 2011). For example, studies on the mosquitoes, *An. albimanus*, *Cx. pipiens pipiens*, and *Cx. quinquefasciatus*, revealed alterations in the Acetylcholinesterase genes, which in turn reduced the binding efficiency with insecticides thereby overcoming insecticidal effects (Liu, 2009). Pyrethroids are well known to exert insecticidal effects by altering the function of voltage-sensitive sodium channels in nerve membranes of many insect species. It has been documented that pyrethroid resistance stems from point mutations in the voltage-gated sodium channels resulting in knockdown resistance, commonly termed kdr (Williamson *et al.*, 1993).

#### **1.1.7.4 Alternative control methods**

Alternative methods in mosquito vectors include among others, breaking the life cycle of the mosquito and population suppression including reducing breeding sites such as draining of rice fields, fish ponds in Java, Indonesia (Takken and Knols, 2009). According to Klayman (1985), the major strategy is treating the disease using drugs such as artemisinin and its synthetic relatives and derivatives as recommended by world health organization as the primary treatment against *P. falciparum* either singly or in combination with other drugs. However it has been found out that artemisinin based treatments are beginning to lose their effectiveness as it is the case in Southeast Asia (Ariey *et al.*, 2014; Winzeler *et al.*, 2014). Currently there is no evidence of artemisinin resistance in Africa and WHO is strengthening its routine of monitoring and surveillance against resistance emergence in the continent (Talisuna *et al.*, 2013).

In Botswana, a combination of strategies for controlling malaria and its vector have been used. These comprise public education about the disease and vector, personal protection measures such as protective clothing and the use of repellents, control of the vector and destruction of sites such as grasses and ponds where the vector rests and breeds respectively (MoH, 2007). The government of Botswana has made efforts in controlling the malaria vector by IRS and the use of ITNs over the years as a national intervention control strategy, and this has been effectively implemented with a success (MoH 2007; Simon *et al.*, 2013). However, according to Ministry of Health (MoH) (2009), one of the most overlooked and under-rated aspect of IRS operation is evaluation and monitoring of the programme. One of the overlooked indicators for operational monitoring and evaluation of IRS includes percentage of structures sprayed, proportion of population protected, spot check of quality of spraying, monitoring insecticide resistance, vector resting density (indoor and outdoor) and the vector night biting densities (indoor and outdoor) (MoH, 2007; MoH, 2009). Wherefore, without these

monitoring indicators, vector control strategies remain a challenge. Therefore there is a need for regular assessment and evaluation to establish indicator baseline thus facilitating appropriate and relevant interventions.

### **1.1.8 Climate change and insect population abundance**

Recent anthropogenic climate change and its effects on biodiversity has been a significant societal challenge (Lineman *et al.*, 2015). According to Urry (2015), climate change is defined as a change in climatic patterns of the global or regional temperatures which is associated with the increase in atmospheric carbon-dioxide. However, global warming is defined as an increase in global temperature patterns over a long period of time (Lineman *et al.*, 2015). The increase in global temperature is a threat to life on earth for both fauna and flora (Thuiller *et al.*, 2005; Hunter, 2007; Sekercioglu *et al.*, 2008). It affects species survival and reproduction, by reducing the potential and available suitable habitats (Beaumont *et al.*, 2002). Although some species may be at risk of extinction, some display evolutionary responses in adapting to the climatic change (Parmesan, 2006). In addition to evolution (across generations), within generation adjustment of physiology e.g. phenotypic plasticity can also enables species to survive and adapt in new environments within short period of time (Chown *et al.*, 2007). Some can however be able to establish over a long time, by means of natural selection (Lee *et al.*, 2007). Physiological traits can be developed, (as an example of phenotypic plasticity) through pre-determined conditions in the laboratory (acclimation) or field trials (acclimatization) (Chown and Nicolson, 2004). It is however, asserted by Nyamukondiwa *et al.* (2010) that species can evolve invasion success due to phenotypic plasticity of thermal tolerance. Furthermore, adaptation and survival of insects in an environment, is enhanced by behavioral thermoregulation activities and mechanisms that depend on solar radiation (Angilletta, 2013). Overall, Chown and Nicolson (2009) suggest that species diversity, richness and thermal tolerance is however, largely influenced by climate variability. Indeed climate change may not only be limited to effects on biodiversity but also to pesticides efficacy in general. They may degrade faster and to some extent contribute to faster resistance in controlled insect species.

#### **1.1.8.1 Insects low temperature tolerance and climate change**

Insects do play an important role in mutual ecological interaction with other species. They can be plant pollinators, a source of food and host for other species to benefit (Lenteren *et al.*, 1987;

Kearns *et al.*, 1998; Van Huis, 2013). Climate change can disrupt these ecological structures (Memmott *et al.*, 2007). They can be due to dispersal in search for suitable resources and habitats (Dowdy, 1994; Walters *et al.*, 2006). Despite global warming, insects are capable of adapting to climatic changes through mitigating mechanisms for their survival (Kingsolver *et al.*, 2011). These strategies make insects to tolerate both low and high temperatures. There are two main survival strategies in which insects tolerate low temperature. They can undergo diapause or quiescence or alternatively remain active during low temperatures (Košťál, 2006; Bale and Hayward, 2010). Quiescence is the suppression of metabolic activities directly due to environmental factors such as temperature, nutrition and moisture while diapause should be restricted to instances where development and activity is arrested spontaneously (Tauber *et al.*, 1986; Lees, 2016). Both adaptive strategies can enable an insect to survive winter or a cold season. All developmental life stage of insects (egg, larvae, pupae and adult) may undergo this physiological arrest. During unfavorably low temperatures, adult (female) mosquitoes of species that diapause may live up to six months to nine months (Kappus and Venard, 1967; Denlinger, and Armbruster, 2014). Mosquitoes that diapause are able to facilitate disease transmission when conditions become conducive for their growth, reproduction and survival.

#### **1.1.8.2 Critical thermal minima (CT<sub>min</sub>)**

Insects that remain active during low temperatures use several strategies to keep on with their survival. At their critical thermal minimum (CT<sub>min</sub>) insects enter chill-coma, a reversible state where neuromuscular transmission and movement cease. Critical thermal minimum knockdown is reached when an insect loses the strength, ability to respond or coordination to stand (Block *et al.*, 1990). Therefore, it is the lowest temperature for an activity (fitness enhancing trait) in insects thus, relating to its performance in a temperature ‘influenced’ ecological setting. According to MacMillan and Sinclair (2011), chill-coma is possibly propelled by an inability to maintain ionic homeostasis through temperature sensitivity on ion-motive ATPases, ion channel gating mechanisms, and/or the lipid membrane, leading to a loss of nerve and muscle excitability. If the period of chilling continues for a prolonged time, this may bring about an irreversible chilling injury to an insect (Košťál *et al.*, 2006). Therefore exposure to benign temperatures make chill-coma a reversible state thus allowing insects to survive after their knockdown. A critical thermal minimum is a significant fitness trait ecologically, as it shows the ecological lowest temperature to activity. This activity may be feeding, mating, dispersal,

mate finding, host finding or any locomotory related fitness trait. Combined with critical thermal maxima, the two show the ecological thermal functional window, the temperature range upon which an organism is active.

### **1.1.8.3 Cold hardiness**

If temperatures are at sub-zero, insects survive low temperatures either by freeze avoidance or freeze tolerance. Freeze avoidance is the ability of an insect to keep body fluids liquid below their ordinary melting point while freeze tolerance, insects strive by surviving the formation of ice in their tissues (Zachariassen, 1985). Freeze-avoiding insects remove ice nucleators that initiate ice formation, synthesizing antifreeze proteins to reduce the nucleation potential of seed crystals, and accumulating sugars and polyols, such as trehalose or glycerol, which also lower the crystallization temperature (Lee, 2012). This is a temperature at which a fluid spontaneously freezes following cooling, known as the supercooling point (SCP) (Renault *et al.*, 2002). It is at this point that membranes are stabilized at low temperatures hence, a survival mechanism (SCP) for insects to avoid freezing. Furthermore, cold tolerant insects may lose water by evaporation when subjected to sub-zero temperatures in the presence of ice (eg. arctic springtails) through process called cryoprotective dehydration as a form of cold hardiness (Sørensen and Holmstrup, 2011). Insects that are sensitive to the direct effects of low temperature are referred to as chill-susceptible (Bale, 2002). When these insects are cooled to low temperatures they enter into a reversible state of neuromuscular paralysis, called chill coma. It is a state of depolarization of the resting membrane potential and a loss of neuromuscular excitability (Andersen *et al.*, 2015). Figure 4 below summarizes cold hardiness responses in insects when cooled.

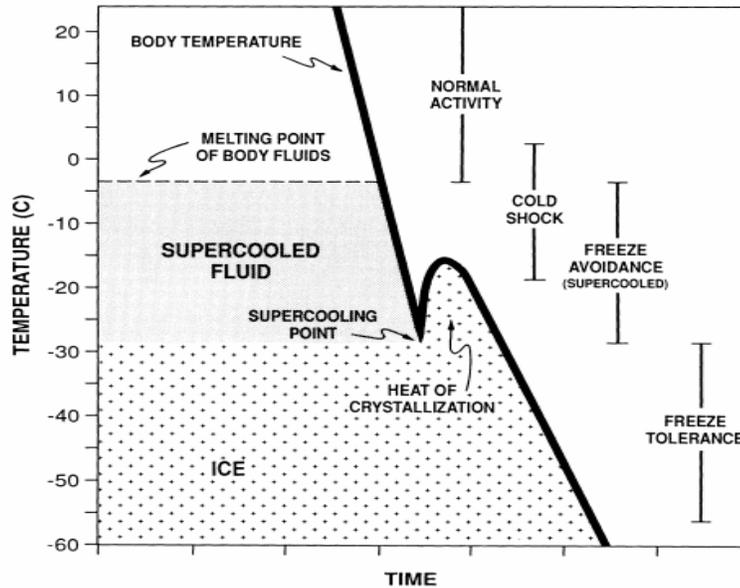


Figure 4: Insect responses when experiencing sub-zero temperatures (Lee, 1989).

#### 1.1.8.4 High temperature tolerance

Extreme high temperatures impose shock, stress, irreversible injury and/or death to an insect. Elevated temperature increases both the metabolism and respiration to the level that threatens an insect's life (Neven, 2000). However, insects have mechanisms in which can become useful in tolerating high levels of temperature. During extreme temperatures, insects lose water thus desiccating them. Insects are able to accumulate trehalose not only in low temperatures but also during dehydration and high temperature. It is known to act as a buffer to prevent unwanted biochemical interactions during the stress period (Watanabe *et al.*, 2002). Production of heat shock proteins is also common with insects undergoing heat shock. These proteins function as molecular chaperones that assist an organism to cope with stress of both an internal and external nature (Sørensen *et al.*, 2003). Plastic responses within generation and across species may improve high temperature tolerance through adaptation (Chidawanyika *et al.*, 2017). That is, both the short term and the long term responses in the environment (DeBiaise and Kelly, 2015).

#### 1.1.8.5 Critical thermal maxima (CT<sub>max</sub>)

Despite the tolerance exhibited by insects against high temperatures, an injury from disrupted membranes may be possible thus causing death (Chown and Nicolson, 2004). The point at which an

insect experiences high temperature that leads to physiological failure and/ death is also known as the critical thermal maximum (CT<sub>max</sub>). As opposed to the CT<sub>min</sub>, it is a measure of the highest temperature for an insect to show activity. Although Terblanche *et al.* (2007) asserts that critical thermal limits depend on the methodological context, CT<sub>max</sub> have been found to be ecologically relevant in the increasing atmospheric temperature which directly affects insects in their habitats (Pörtner, 2001). Hence, it gives a representation of an insect performance at high temperature relative to its natural ecological microhabitat. Insects (such as mosquitoes) whose life cycle is adversely affected by temperature, need assessment of CT<sub>max</sub> and CT<sub>min</sub> in the climate change scenarios. Critical thermal maximum remains highly significant ecologically, as it indicates the maximum temperature that can allow fitness activities for any insect species. Coupled with CT<sub>min</sub>, they determine the thermal window for any insect activity.

### **1.1.9 Malaria vectors and climate change**

Climate change and vector borne disease emergence have been associated with increased atmospheric temperatures and rainfall leading to increased morbidity and mortality (Harvell *et al.*, 2002). According to Tonnang *et al.* (2010), there is a potential for redistribution of malaria vectors in Africa due to climate change. The establishment of mosquito vectors in new location is a threat of disease emergence in an area that was previously declared disease free (Zell, 2004). Therefore there is a need for continuous monitoring of vectors in different geographic locations as a way of arresting the spread of malaria, other vector- borne diseases and detecting new vector species and associated pathogens thereof.

### **1.2 Statement of the problem**

Botswana is gearing towards malaria elimination by 2018. It is amongst the four countries (Botswana, Swaziland, South Africa, and Namibia) in Southern Africa certified by WHO on a campaign of zero malaria transmission. Currently, Botswana uses two main national strategies on vector- parasite approach to control the disease. The disease diagnosis and parasite control by drugs is so far a success story in the country. Vector control approaches by the use of IRS and distribution of insecticide impregnated bednets have over the years become a key intervention strategies in minimizing *Plasmodium* parasite transmission to humans. The continuous use of these insecticides might have impacted the mosquitoes- insecticide sensitivity. The local population receiving these

interventions, always complains of mosquito aggressiveness and high densities after mounting the strategies (IRS and ITNs). As such, no study has been done to explain the mosquito post-exposure to insecticides. Hence the bioassays (on insecticides susceptibility) will be carried out to establish a baseline on insecticides- mosquito interaction dynamics. Furthermore, in the efforts to control the vector, there are some environment- vector related factors that contribute to malaria dynamics becoming a challenge to eliminate. Prior to malaria elimination target by 2018, the government of Botswana had to postpone the 2015 malaria elimination strategic plan due to escalating malaria cases and related deaths detected every year. It is not yet clear in Botswana if both the biotic and abiotic factors and vector-pathogen physio-ecological adaptation of the vector, have a correlation to malaria prevalence. These factors make malaria control strategies highly problematic and thus creating a continuous burden to the society. Therefore, it is important to further assess potential factors impacted by pressure from insecticides used for IRS and LLINs, water bodies and mosquito density factor that may support vector breeding and the eco-physiological adaptations responses of the Anopheline species due to climate change.

### **1.3 Justification and significance of the study**

The key malaria intervention strategies revolve around the use of insecticides for IRS and LLINs. The monitoring and evaluation of susceptibility levels of the local malaria vectors in the endemic area is essential. This enables for proper and timely intervention approaches on vector resistance. The MoH may also be informed better on alternative vector control insecticides recommended by WHO. This study is also anticipated to provide mosquito diversity and density with regard to their association with natural water bodies in malarious districts. The densities may assist in prioritizing vector intervention strategies to minimize potential Anopheline vectors in certain localities. This study will as well sensitize on thermal biology of the Anopheline mosquitoes, to help explain how temperature tolerance likely shape the population phenology, activity and abundance of the malaria vectors through investigating critical thermal limits to activity (CTLs), for the key vector species. The CTLs information is essential in evaluating how mosquitoes can cope with temperature fluctuations. This critical information is currently lacking, for the malaria endemic areas of Botswana. This work will thus bridge these gaps with the aim to ameliorating issues of vector control towards malaria elimination.

#### **1.4 Research Questions**

1. Do natural water bodies support the breeding and the abundance of mosquitoes?
2. Is *An. arabiensis* resistant to the insecticides?
3. What are the critical thermal limits to activity for *An. arabiensis*?

#### **1.5 General Objectives**

1. To determine the abundance of the mosquitoes around the natural water bodies.
2. To assess mosquito susceptibility to currently registered insecticides.
3. To determine the critical thermal limits of the *An. arabiensis*.

##### **1.5.1 Specific Objectives**

- 1a. To determine mosquito diversity around natural water bodies in three malaria endemic districts.
- 1b. To determine mosquito abundance at different distances from water bodies
- 1c. To determine the abundance of females versus males in three malaria endemic districts
  
- 2a. To assess *An. arabiensis* susceptibility levels to four classes of insecticides
- 2b. To determine *An. Arabiensis* knockdown time (KDT<sub>50</sub>) to different insecticides
  
- 3a. To determine the effect of sex on critical thermal limits in adult *An. arabiensis*.
- 3b. To determine the effect of developmental stage on critical thermal limits in *An. arabiensis*.

#### **1.6 Hypothesis**

1. Mosquito abundance is the same at different distances away from the natural water bodies (rivers).
2. *An. arabiensis* is susceptible to currently registered insecticides.
3. The critical thermal limits to activity of *An. arabiensis* larvae and adults are not the same.

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## **CHAPTER TWO**

**Mosquito (Diptera: Culicidae) diversity and abundance around permanent natural water bodies in malaria endemic districts (Okavango, Ngamiland and Bobirwa): implications on malaria vector population dynamics.**

## 2.0 Introduction

Mosquitoes are insects belonging to family Culicidae with approximately 41 genera consisting of about 3500 species (Becker *et al.*, 2010; Wilkerson *et al.*, 2015; Nava and Debboun, 2016). Of all the genera, only four (*Aedes*, *Culex*, *Anopheles* and *Mansonia*) are known to be of medical importance (Rueda, 2008). They serve as primary, secondary and/ or bridge vectors of pathogens that cause diseases to both humans and animals (WHO, 2014). Several pathogens are causative agents of diseases such as malaria (*Plasmodium*, *Haemoproteus*), filariasis (worm- *Wuchereria bancrofti*), yellow fever (virus), dengue (virus), chikungunya (virus), zika (virus), Japanese encephalitis (virus) and hemorrhagic fever (virus) (Manguin *et al.*, 2010; Shah *et al.*, 2016; De Wispelaere *et al.*, 2017; Shragaiet *et al.*, 2017). The female *Anopheles* mosquito is the one that is able to transmit the parasite (*Plasmodium*) to its host with *Plasmodium falciparum* being the main pathogen of malaria vectored mainly by siblings of the *Anopheles gambiae* complex and *Anopheles funestus* group (Gething *et al.*, 2016; Wiebe *et al.*, 2017).

The reproduction and the survival of mosquito vectors and many other insects are influenced by both the biotic and abiotic factors of the environment (Ginsberg *et al.*, 2017). Climatic conditions play a vital role in population dynamics of mosquitoes (Farajzadeh *et al.*, 2015; Marini *et al.*, 2016). Temperature and relative humidity fluctuations have become a key factor on mosquito population dynamics (Dickerson, 2007; Beck-Johnson *et al.*, 2017). Habitats are as well important in the breeding, diversity, abundance and distribution of mosquito populations (Marquetti-Fernández *et al.*, 2017). Mosquitoes breed in various aquatic habitats such as stagnant waters, flowers, artificial containers, tree and rock crevices, temporary and permanent pools (Mattah *et al.*, 2017). Domestic structures e.g. homes also contribute to the breeding of mosquitoes in habitats such as roof gutters, flower pots and vases, containers (tins, packaging disposables, old tyres and others), gardens and lawns (irrigation) and drainage systems (Banerjee *et al.*, 2013; Getachew *et al.*, 2015; Naz and Shabnam, 2014). Therefore, vector abundance becomes persistent in areas with such breeding microhabitats and where feeding and resting refugia is present. Adult mosquitoes can communicate with the environment through chemical and mechanical stimuli in locating hosts, oviposition and resting sites (Brugman, 2016; Perea and Callaghan, 2017; Wenseleers and van Zweden, 2017). The search for such 'resources' and 'hotspots', may lead to mosquito dispersal within a geographical location. However, mosquitoes are not strong flyers as such, the dispersal is constrained (Ciota *et al.*, 2012). According to Medeiros *et al.* (2017), mosquito dispersal generally remains within two kilometers from their natal breeding habitats.

However, dispersal may be passively assisted by wind, and movement of distances in excess of about 100 km or more from breeding sites have been reported (Service, 1997). Regardless of the economic significance of mosquitos and malaria transmission in Botswana, their population dynamics with respect to biophysical factors has not been elucidated in the malaria endemic districts of the country. Moreover, availability of permanent water bodies is a significant contributory factor to vector abundance (Pires and Gleiser, 2010; Rey *et al.*, 2012; Su *et al.*, 2016), but nevertheless, the role of large water bodies and vector population abundance is largely unknown for Botswana. Therefore, it is unclear to tease apart the role of large water bodies or micro-breeding environments (tins, small ponds, containers, tires and others), in mosquito abundance. This has a bearing in vector control strategies for different endemic districts with unique permanent large water bodies (rivers).

This research was therefore aimed at (i) determining mosquito diversity and abundance in three malaria endemic districts, (ii) determining mosquito densities at different distances from permanent water bodies and (iii) investigating the relative proportion of female versus male mosquitoes.

## **2.1 Materials and methods**

### **2.1.1 Study area**

The study was conducted in three districts (Okavango, Ngamiland and Bobirwa) in Botswana. These were amongst the six malaria endemic districts/ sub-districts (Chobe (S17. 7952°, E025. 1711°), Ngamiland (S19. 9953°, E023. 4181°), Boteti (S21. 0300°, E024. 3995°), Tutume (S20. 5026°, E027. 0441°, Okavango (S18. 3673°, E021. 8390°) and Bobirwa (S22. 0940°, E028. 5798°) situated in the northern part of the country (see Fig. 1).



Figure 1: Map of Botswana showing malaria endemic districts used for sampling in this study (Modified from CSO, (2008). (Okavango: 1, Ngamiland: 2, Chobe: 3, Boteti: 4, Tutume: 5 and Bobirwa: 6).

## 2.1.2 Study design

### 2.1.2.1 Mosquito sampling

Adult mosquitoes were trapped using Centre for Disease Control (CDC) light traps (Model: 512; John W. Hock Company, Florida, USA) ( $\text{CO}_2$  baited) along the river in three study districts (Okavango (S18. 16625°, E021. 47330°), Ngamiland (S20, 01830°, E023, 24501°) and Bobirwa (S22. 17874°, E028. 86266°). The trapping was repeated over three days per sampling time and repeated over two seasons during 2015/ 2016 and 2016/ 2017 malaria transmission season and done between December and March. During trapping, the lights were put in four different positions from the river (Embankment, 50m, 100m and 3.5km) (Fig. 2). The 3.5km distance was the furthest position from the sampled river in which mosquitoes would normally not migrate or move beyond. Mosquito species have been found to generally disperse within two-kilometer radius from larval habitats (Hamer *et al.*, 2014; Medeiros *et al.*, 2017). The distance between each row of light traps (river length wise) to another was 50m. The traps were set in the evenings (6 O'clock) and checked in the morning (6 O'clock) to give a 12 hour collection period.

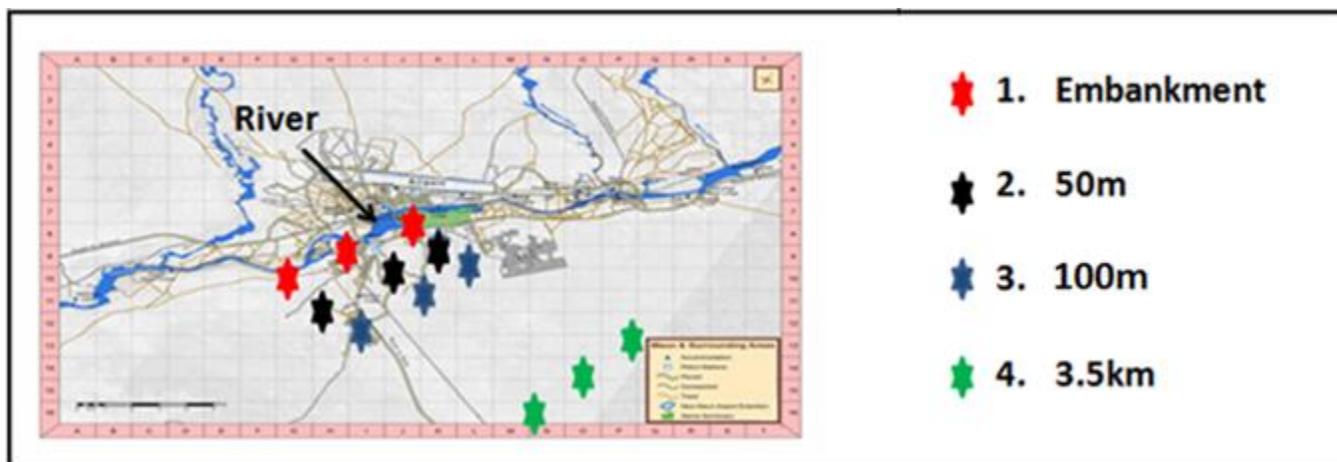


Figure 2: Modeling of the river distance pattern mosquito trapping (Modified from Ngami Data Services: <http://www.nds-printingbotswana.com>).

#### 2.1.2.2 Sample storage

Following collection, adult mosquitoes were put in a container with dry ice for about 10 minutes to kill them. The specimens (batch of 20) were then put in 25 mL vials perforated on the lid. The vials with specimen were put in an air tight zip lock containing 50g of silica gel (Glassworld: Orange particle size- 2,8 to 7.0mm, South Africa). The specimens were kept until further processing in the laboratory.

#### 2.1.2.3 Sample analysis

The mosquito samples were identified to genus level (*Culex*, *Aedes*, *Anopheles*, *Mansonia* and others) using gross morphology (Gillies and Coetzee, 1987), by employing the microscope (Model: BS-3060BT, BestScope International Ltd. Beijing, China). The identification was based on important mosquito parts such as the antennae, palps, thorax, wing venation, legs and the abdomen. The mosquitoes were again sexed based on differences in their antennae (plumose: males, pilose: females).

#### 2.1.2.4 Statistical analysis

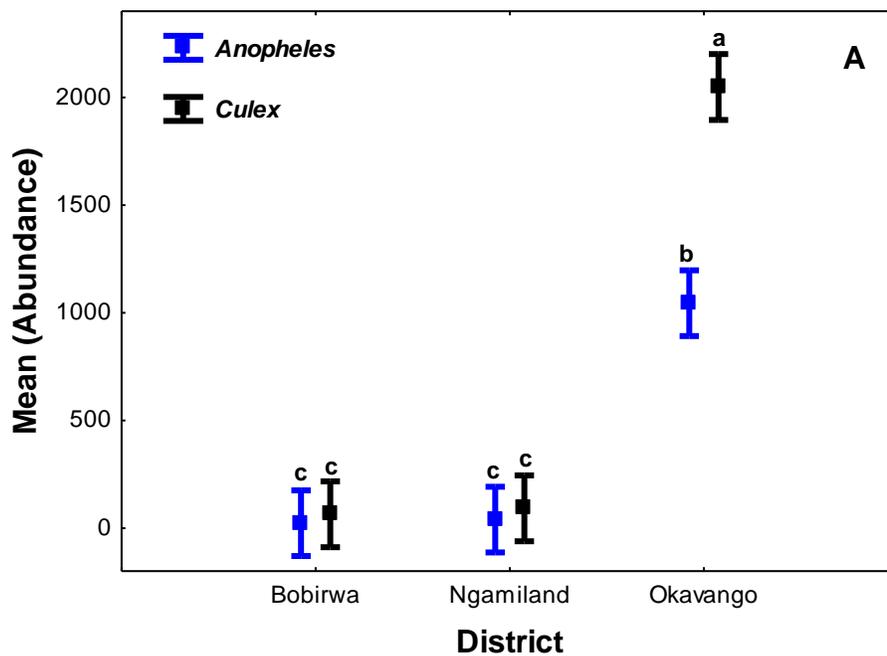
Statistica 13.2 (Dell Inc. Tulsa. USA) was used in analyzing the data. Data was checked for normality using Shapiro Wilk test. First, repeated measures analysis of variance ANOVA was used to look into the effects of day (over the three day collection period) on mosquito abundance for the three districts sampled. However, day did not have an effect on mosquito abundance, so for subsequent

analysis, data from the three days were pooled together. Factorial ANOVA was used to analyze the interactive effect of mosquito densities with distance from water bodies, genera and sex in different endemic districts (Bobirwa, Ngamiland and Okavango). One way ANOVA was further used to analyze the densities of *An. gambiae* complex and *An. funestus* group. Turkey Kramer's posthoc tests were used to separate statistically heterogeneous groups.

## 2.2 Results

### 2.2.1 Mosquito diversity and abundance

There was a statistically significant difference in mosquito abundance across the three endemic areas (Fig. 3A; Table 1) and for the two genera *Anopheles* and *Culex* (Fig. 3A; Table 1). Okavango had significantly higher genera abundance across the sampling sites, and the abundance between *Anopheles* and *Culex* were statistically different for this district. Nevertheless, the abundance of these two genera was not statistically different for the other two districts Bobirwa and Ngamiland (Figure 3A). The district x genera interaction effect was also highly significant (Fig. 3A; Table 1). Out of all the districts, Okavango was significantly higher ( $F_{(1,4)} = 1451, p < 0.0001$ ) Anopheline species belonging to the *An. gambiae* complex and *An. funestus* group (Fig. 3B), thus, sibling species of the *An. funestus* group were fewer than the *An. gambiae* complex.



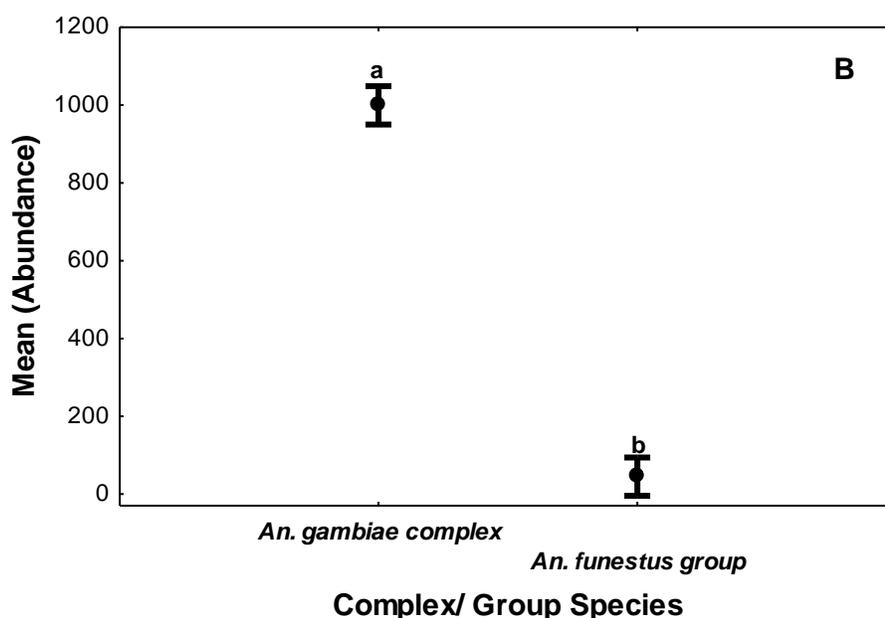


Figure 3: *Anopheles* and *Culex* mosquito diversity and abundance in Bobirwa, Ngamiland and Okavango (A) and density proportions of *An. gambiae* complex and the *An. funestus* group in the Okavango district (B). Each point represents mean  $\pm$ 95% CL. Means with the same letter are not statistically different.

Table 1: Factorial ANOVA summary results of mosquito abundance and diversity for malaria endemic districts.

Effect	F- value	P- value
District	F(2,12)= 302	<0.0001
Genera	F(1,12)= 41	<0.0001
District X Genera	F(2,12)= 31	<0.0001

### 2.2.2 Mosquito densities with distance from the river.

Mosquito abundance was significantly higher on the embankment (by the river bank) with generally reduced abundance away from the river (Fig. 4; Table 2). Okavango had significantly higher numbers while Bobirwa had the least (Fig. 4; Table 2). There was a significant difference in abundance between *Anopheles* and *Culex* mosquito species for the three districts (Fig. 4; Table 2) with *Culex*

showing the highest densities for all the districts. The interactions for district x genera (Fig. 4; Table 2), district x position (Fig. 4; Table 2), genera x position (Fig. 4; Table 2) and district x genera x position (Fig.4; Table 2) were also highly significant.

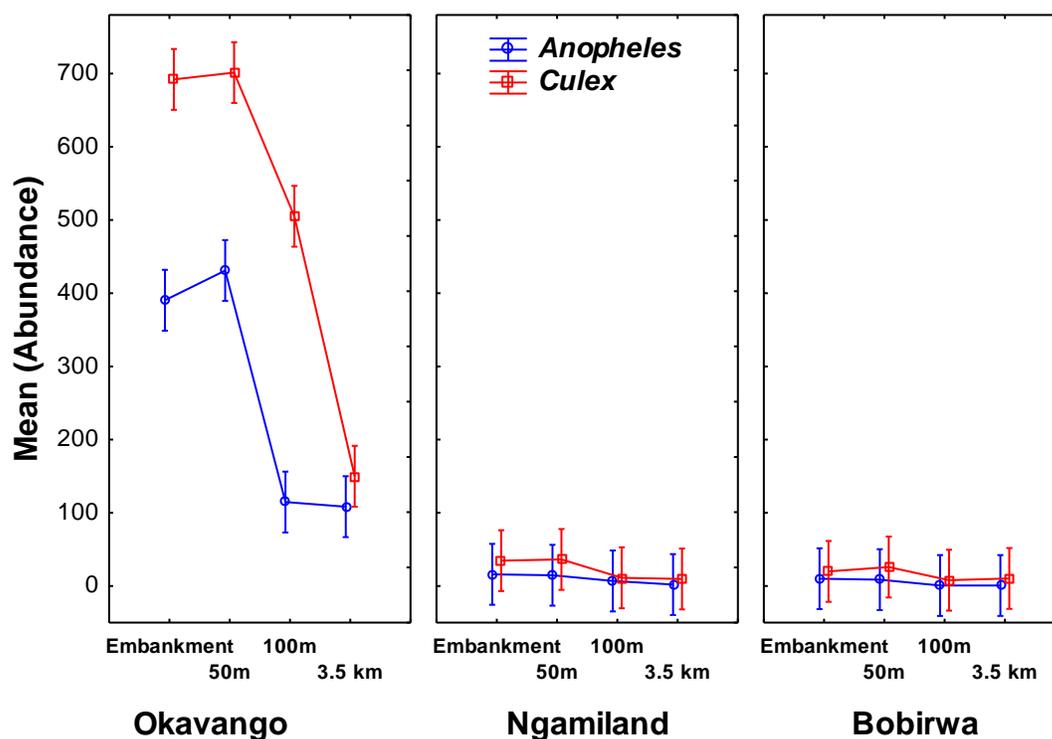


Figure 4: Summary results on mosquito abundance with distance from permanent water bodies (rivers) in malaria endemic districts. Each point represents mean  $\pm$ 95% CL.

Table 2: Factorial ANOVA summary results of mosquito abundance at different distances from permanent water bodies (rivers).

Effect	F- value	P- value
District	F(2,48)= 864	<0.0001
Genera	F(1,48)= 118	<0.0001
Position	F(3,48)= 78	<0.0001
District x Genera	F(2,48)= 89	<0.0001
District x Position	F(6,48)= 62	<0.0001
Genera x Position	F(3,48)= 8.7	<0.0001
District x Genera x Position	F(6,48)= 31	<0.0001

### 2.2.3 Mosquito sex density proportions.

There was a statistically significant sex difference (Fig. 5; Table 3) for *Anopheles* and *Culex* species, with Okavango being very distinct. Generally males were consistently fewer than females for all the districts with densities of *Culex* (male versus female) higher than that of *Anopheles*. Furthermore, there was a highly significant difference (Fig. 5 Table 3) at district level. District x genera and district x sex were highly significant (Fig. 5; Table 3) whilst genera x sex and district x genera x sex interactions were not significant (Fig. 5; Table 3).

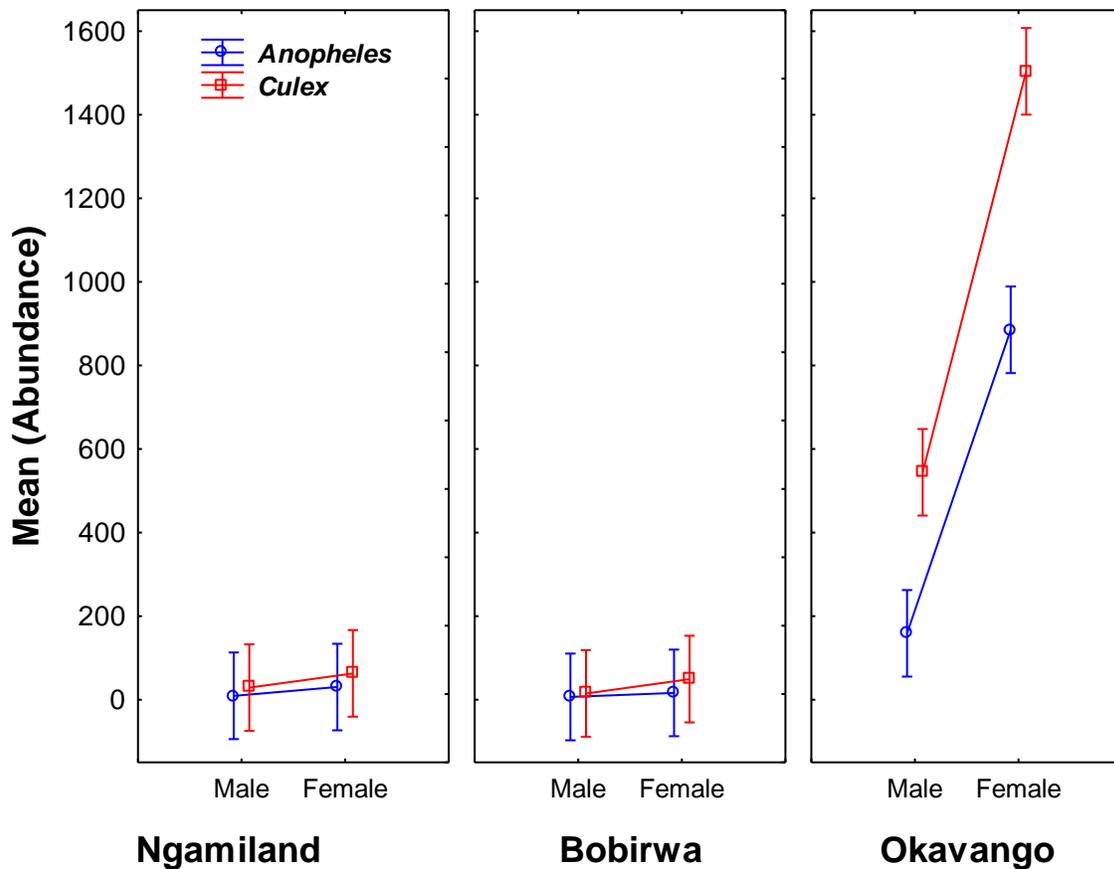


Figure 5: Mosquito abundance proportion of female versus male in malaria endemic districts. Each point represents mean  $\pm$ 95% CL.

Table 3: Factorial ANOVA summary results of mosquito proportions (female versus male) in malaria endemic districts.

<b>Effect</b>	<b>F- value</b>	<b>P- value</b>
District	F(2,24)= 294	<0.0001
Genera	F(1,24)= 40	<0.0001
Sex	F(1,24)= 105	<0.0001
District x Genera	F(2,24)= 30	<0.0001
District x Sex	F(2,24)= 89	<0.0001
Genera x Sex	F(1,24)= 2.4	=0.132
District x Genera x Sex	F(2,24)= 1.5	=0.238

## 2.3 Discussion

### 2.3.1 Mosquito diversity and abundance

*Anopheles* and *Culex* mosquito species were reported in all the three districts sampled. The geographic distribution of mosquito species is influenced by several factors that directly or indirectly affect their growth, survival and reproduction (De Souza *et al.*, 2010; Weicheld, 2015). Ezihe *et al.* (2017a) affirmed that mosquito diversity, species composition and abundance may be influenced by seasonal distribution, microclimatic factors (temperature, relative humidity, precipitation) and landscape features (vegetation, water bodies, impervious surface cover). While availability of breeding sites is the main determinant factor (Mattah *et al.*, 2017), Zित्रा *et al.* (2017) suggested that, landscape and the ground cover also plays a critical role in the distribution and abundance of mosquitoes. Vegetation supports resting mosquitoes and thus survival and may likely be different across the three sampled districts. Okavango reported the most abundant species of *Culex* and *Anopheles* than Ngamiland and Bobirwa. This could be due to more stable and permanent water bodies and larger vegetation diversity in the Okavango basin compared to the other two localities. Moreover, some researchers associated climate variability and landscape heterogeneity as the main important factors that could contribute to diversity and abundance of mosquitoes (Chaves *et al.*, 2011). Though climate variability was not measured for this study in establishing mosquito abundance correlation in the districts, it remains an important factor that can be investigated in the future including other factors to account for the diversity. Also, mosquito abundance and diversity may possibly be shaped by the specific type of vegetation available to provide dietary requirements and resting behavioral needs. That

is, some plants naturally secrete compounds that deter mosquitoes thus making a certain area inhabitable. Overall, *Anopheles* and *Culex* as vectors of pathogens, pose a risk of potential emergence and transmission of diseases in districts where they were found especially where the abundance was high such as in Okavango. This was demonstrated by Tangena *et al.* (2017) on the risk of disease through exposure to potential vector mosquitoes. Thus, there may be a greater malaria risk for Okavango relative to other malarious districts of Botswana, and thus control efforts should also be aligned with this data.

*Aedes*, *Mansonia* and other mosquito genera were not recorded in this study. Although, it is theoretically known that the Centre for Disease Control (CDC) light attracts a wide range of mosquito species, it was however, not as effective in this study. Some studies have therefore shown that *Aedes* cannot be effectively trapped by CDC light but instead the BG Sentinel™ (BGS) traps (Gibson-Corrado *et al.*, 2017). Furthermore, a maximum capture is attained by a black colour enhancement on the trap itself (Iyaloo *et al.*, 2017). Moreover, *Aedes* species are highly affected by diurnal temperatures as demonstrated by Liu-Helmersson *et al.* (2016). Though *Mansonia* may be less studied, it is a mosquito genus of medical importance (Ughasi *et al.*, 2012). However its diversity in the study sites (districts) was not reported. Researchers have found out that *Mansonia* larvae thrives better in submerged vegetation or swampy forests, on which the aquatic larvae attaches, thus sucking plant juices for its growth and development (Chang *et al.*, 1988; Krishnamoorthy *et al.*, 1994). It is therefore, assumed that ‘vegetative’ swampy breeding habitats and specific host plants (see Chandra *et al.*, 2006) needed by the immature larval stage of *Mansonia*, may have been lacking in the study sites for supporting the breeding and establishment of a significant population. Nevertheless, future studies need to be done to look into the abundance of these species in the country, with a view of establishing the risk of its associated parasites and diseases.

*Culex* was found abundant in all districts. This could have been linked to its diverse and explored breeding sites ranging from clean to polluted water sources (Ebuka *et al.*, 2017b). However, according to Kengluocha *et al.* (2005) breeding of *Anopheles* is adversely affected by the quality of breeding water habitats. It may therefore, be this behavioral selectivity on breeding sites that contributed to its lower densities than *Culex* species. Though recently, Gunathilaka *et al.* (2013) demonstrated the potential for *An. culicifacies* to breed (through adaptation) in polluted water sources

due to less availability of clean preferred breeding waters. Among the *Anopheles* species, the *An. gambiae* complex was reported abundant compared to the *An. funestus* group. Although *An. funestus* species were known to be highly sensitive to insecticides, recently there are reports on the rise and increase in insecticide resistance (Casimiro *et al.*, 2006) including multiple resistance (Riveron *et al.*, 2016). Therefore, it was assumed that over the years, *An. funestus* siblings were susceptible to insecticides used for indoor residual spraying (IRS) in endemic districts, reducing its abundance and diversity. Indeed, there is a need to confirm its susceptibility status to insecticides in verifying the assumption. The siblings of the *An. gambiae* complex are difficult to control in that they have developed a behavior to feed and rest both indoors and outdoors thus enabling them to diversify (Faye *et al.*, 1997; Reddy *et al.*, 2011) and escape control strategies'. According to Wiebe *et al.* (2017), *An. gambiae* complex and *An. funestus* group are currently the most important vectors of malaria in sub-Saharan Africa. Therefore, there is a need to further investigate their sibling species diversity (genetic) and geographical distribution across the country with implications to vector risk capacity. Furthermore, since mosquito diversity and abundance is likely shaped by landscape and climate heterogeneity (Chaves *et al.*, 2011; Roiz *et al.*, 2015), it may also follow there may be differential pesticide susceptibility for the same species, associated with differences in selection pressure and localized climatic conditions and landscapes. Thus, a more fruitful area of study would also be looking into localized resistance assays within and across mosquito species.

### **2.3.2 Mosquito densities with distance from the rivers.**

The *Anopheles* and *Culex* mosquitoes were reported abundant close to water sources and the densities reduced with distance. The most important influencing factor attributing to this abundance could be the role of natural water bodies in vector breeding (as in e.g. Pires and Gleiser, 2010). Mosquitoes breed in different types of water bodies, and in a wide range of larval habitats such as temporary, permanent, sunny, shaded stagnant and slow moving water sources (Hamza, 2016). Breeding can also take place in water that collects in animal hooves around the water sources thus maximizing breeding and high densities around natural water sources (Chirebvu and Chimbari 2015; Dida *et al.*, 2015). Animals that come to drink from water bodies do not only serve to create hoof prints (as larval water habitat) by the water bodies but attract mosquitoes as hosts for blood meal thus, maintaining mosquito population densities and survival rates on the embankment (Phasomkusolsil *et al.*, 2013). Furthermore, mosquitoes may device alternative blood feeding behavior on human hosts as

they frequently visit the river embankment for activities such as swimming, washing and fishing thus enhancing the population densities and stability around these areas. Thankachan and Gopinath (2017) described that the diversity of mosquito species in plantation areas and vegetation around water sources attract plants suitable for producing nectar that is needed by both female and male mosquitoes for energy generation. These plants create essential refugia for mosquitoes to rest during egg maturation period hence influencing their physiology and enhance longevity (see Arum *et al.*, 2016). In contrast, these resources may be limited in far distances (such as 3.5km from embankment) from natural water sources leading to low densities. Hence, landscape cover may be the contributing factor in this case as affirmed by Norkute (2014). Furthermore, it had since been established that humidity plays a vital role in mosquito survival (Costa *et al.*, 2010). The natural water bodies may, in this case, provide suitable relative humidity to mosquito microhabitats close by. As such, it is important for vector control and management strategies to prioritize on zones which are at close to water bodies where high vector abundance may lead to ‘successful infections.’ Moreover, since mosquitoes do not disperse >2km (Ciota *et al.*, 2012; Medeiros *et al.*, 2017), human settlements should be avoided within at least 2km of the river embankments, to avoid incidence of large mosquito populations that may impact on malaria epidemics. Nevertheless, rivers and large water bodies form a significant sphere of influence for many rural communities through their direct and indirect resources (Mosepele and Bokhutlo, 2015; Tubatsi *et al.*, 2015). Thus, enforcement of settlements outside this buffer zone may be constrained. Thus, though settlement policies, this research may advise on settlement policies for malarious areas and their relationships with large natural water bodies.

### **2.3.3 Mosquito sex density proportions**

The districts showed a significant difference for mosquito sex (male versus female). However, the interaction of genera x sex and district x genera x sex interactions were not significant. The reason for this sex difference may be low mosquito abundance in Ngamiland and Bobirwa hence difficult to effectively establish the differences in a population as (for instance) shown by Okavango where densities were highest. Secondly, sex determination in mosquitoes could result as a matter of chance controlled for example by a Yobgene in the malaria mosquito (*An. gambiae*) located on the male-specific Y chromosome and regulates the development of a mosquito into being a male (Bernardini *et al.*, 2014). Furthermore, according to McPhatter and Gerry (2017), trapping mosquitoes with CO<sub>2</sub> did not only increase the catches but also increased female catches consistently more than the males. That

is, CO<sub>2</sub> attractant remained biased to the female mosquitoes due to feeding preferences. Furthermore, this may well, be associated with the odorant binding proteins (OBPs) and chemosensory proteins (CSPs) found in the antennae of males and females of mosquitoes for transferring semiochemicals to the membrane-bonded receptors, thus contributed to the activation of olfactory receptor neurons (Peng *et al.*, 2017). Carbon dioxide as a stimulant binds to these proteins and lures them (mosquitoes). According to Taparia *et al.* (2017), female *Culex quinquefasciatus* blood meal feeding behavior induced theregulation of the chemosensory gene for the production of CSPs in host recognition. Hoffman *et al.* (2012) demonstrated with female *An. gambiae*, an increased messenger ribonucleic acid (mRNA) transcript accumulation of OBP2 in the antennae as compared to the male. Therefore, the high density of captured females than males probably showed a 'female mosquito role' in reproduction and desperation in egg development (by seeking proteins from a blood source). Though Bobirwa and Ngamiland showed no sex differences for the species recorded here, while Okavango showed marked differences across sexes. This result has an implication on biting behavior, as an environment of more 'female- biased population' has potential of disease- pathogen transmission at a higher rate by both *Culex* and *Anopheles* mosquitoes.

## 2.4 Conclusion

In conclusion, it was noted that the two genera (*Anopheles* and *Culex*) were found in all the districts with *Culex* having higher densities which could possibly contribute as vectors to pathogens they associate with hence leading to more malaria cases (*Anopheles*) and emerging diseases (from *Culex*). Also that, Okavango had the highest mosquito densities thus important in prioritizing vector control measures not only to individuals living close to the rivers or natural water bodies but to areas with high vector densities. Furthermore, all the districts showed a trend of more females attracted to the trapping light (CDC) than males though, could be much related to the biased trapping technique used.

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## **CHAPTER 3**

**Biological efficacy of *Anopheles arabiensis* (Diptera: Culicidae) control  
by insecticides: implications on resistance management.**

### 3.0 Introduction

Over the years, mosquito vectors and other arthropods have been controlled by the use of synthetic insecticides with dramatic reduction of insect vector-borne diseases and their associated risks (Fontaine, 1980; Hougard *et al.*, 2002; Cooper and Dobson, 2007; Himeidan *et al.*, 2012). However, alongside with the ‘benefits,’ there has been a changing role in insecticides (Metcalf, 1980), with notable pesticide resistance in time and space (Busvine, 1972; Georghiou and Mellon, 1983; Liu, 2015; Marcombe *et al.*, 2017). The injudicious insecticide use, motivated by increased vector borne disease burden (Rowe *et al.*, 2006), mounted pressure on mosquito genetics and through natural selection and evolution (Raymond and Marquine, 1994; Labbé *et al.*, 2007), has caused a drift towards resistance development (Kodandaram *et al.*, 2017). Moreover, pesticides may endanger non target organisms such as terrestrial and marine or freshwater flora and fauna (Ware, 1980; Abbas *et al.*, 2015). Thus, this necessitates the need for more environmentally friendly control alternatives such as ‘softer’ and botanical biopesticides (Khater, 2012). There are four most common organic synthetic insecticides that are capable of conferring resistance to insects, namely the chlorinated hydrocarbons, organophosphates, carbamates and pyrethroids (Zhu *et al.*, 2014). These insecticides are target site specific with organophosphates and carbamates inhibiting activity of the neuro-synaptic enzyme (Acetylcholinesterase) whilst the organochlorines and the pyrethroids target the sodium ion channels (Tiwari *et al.*, 2012; Zhang *et al.*, 2016). Mosquitoes, for example *Anopheles albimanus*, *Culex pipiens pipiens*, and *Culex quinquefasciatus* have shown alterations in the Acetylcholinesterase genes, with consequent reduction in the binding efficiency with insecticides and hence efficacy (Liu *et al.*, 2009). It is as well documented that organochlorines and pyrethroid resistance emanates from point mutations in the voltage-gated sodium channels resulting in knockdown resistance (kdr) (Williamson *et al.*, 1993). Cross resistance as a consequence, may occur when a resistance mechanism, which allows insects to resist one insecticide, also confers resistance to another insecticide (Bregues *et al.*, 2003). Furthermore, it may be possible to occur between insecticides from different chemical classes (Ranson *et al.*, 2011). Moreover, evidence of multiple resistance in malaria vectors has been reported worldwide (Edi *et al.*, 2011; Djouaka *et al.*, 2016). In Africa, insecticide resistance is reported as a worsening situation that needs urgent intervention for effective control of the vector and combat malaria burden (Ranson and Lissenden, 2016). In Botswana, lambda- cyhalothrin (pyrethroid) and Dichloro Diphenyl Trichloroethane (DDT; organochlorine) are the main insecticides used in painted walls and mud and/or unplastered walls respectively. However since 1950s, DDT has been used in the

country for IRS and later complimented by pyrethroids (Ministry of Health [MOH], 2012). Moreover, the pyrethroids are as well used in the insecticide impregnated bednets distributed in the country (Simon *et al.*, 2013). However, the efficacy of these insecticides, in time and space is currently unknown in Botswana.

Pesticides generally have a detrimental effect on the environment where they are used, due to their long term residual persistence and acute fatal effect on non-target beneficial plant and animal species (Iyaniwura, 1991); Zacharia, 2011). They have so far contaminated ground and surface water sources, food products, air, soil thus directly or indirectly affecting human health resulting in cancers, skin and respiratory diseases (Nicolopoulou-Stamati *et al.*, 2016). Moreover, the synthetic pesticides have become ineffective of late due to current insecticide resistance trends (Gnanguenon *et al.*, 2015). However, there are alternative methods that may potentially minimize environmental and human risks from pesticides at the same time combating malaria. The use of biological methods, botanical pesticides, recent biochemical, immunological and genetic techniques may possibly be incorporated as an integrated approach to manage vectors and resistance (Dobrokhotov, 1991; WHO, 2014). As such, the insecticides susceptibility bioassays on malaria vectors would determine resistance level that may necessitates appropriate intervention strategy.

Currently, no work has been done on assessment and monitoring of mosquito vector responses to currently used insecticides in the country. This is regardless of Botswana having been using these insecticides for >60 years (WHO, 2012; Chihanga *et al.*, 2016), a timing that may promote resistance development. Therefore, this work aimed at determining a baseline assessment on insecticide resistance for currently used pesticides in Botswana. The objectives of the study were to (i) assess *An. arabiensis* susceptibility levels to four classes of registered insecticides and (ii) determining its knockdown time (KDT<sub>50</sub>) to the different insecticides which may be essential in future strategic evaluation of insecticide selection and overall integrated management of insecticide resistance.

### **3.1 Materials and Methods**

#### **3.1.1 Larval sampling**

The *Anopheles* larvae were collected from stagnant pools (Bobirwa: S22. 17166°, E028. 91298°, Ngamiland: S20. 01830°, E023. 24501° and Okavango: S18, 17043°, E021, 50967°) in the summer season 2015/ 2016 and 2016/ 2017 between February to March. The larval sampling consent was obtained from the Ministry of Health (Permit: PPME 13/18/1 PS V (353) and community leaders

from sampled areas. The collection was done from morning until noon (0800hrs to 1200hrs) during a clear daylight (Fig. 1A) for effective visualisation of the larvae on the water surfaces. When approaching water sources, precautions were taken not to disturb the larvae and the shadow was always away from the water source as it disturbed the larvae. The collection was done with a larval scoop and a larval tray (Fig. 1B) then transported in netted plastic containers placed in cooler boxes to the laboratory for further processing.

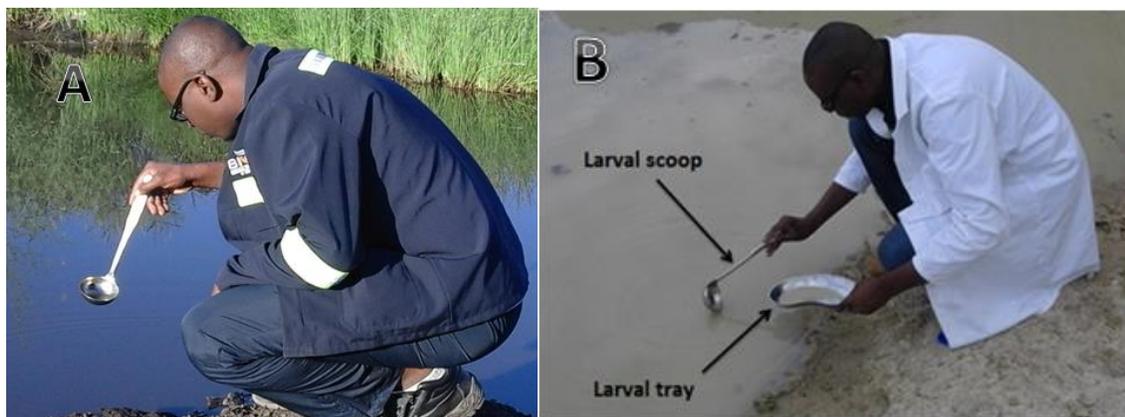


Figure 1: Larvae sampling during daylight at Lechana pond (Bobirwa) (A), larvae collection using a scoop and a tray in Ngamiland (B).

### 3.1.2 Rearing to adult mosquitoes

The mosquito larvae collected from different district water sources were sieved carefully through a strainer and placed onto another container with clear river water from the sampled sites in maintaining their natural habitat *in situ*. The container was covered with a net that allowed gaseous exchange for effective aeration of the mosquito life stages. The larvae were fed with a scoop of fish food (Sera: Vipar family) daily and the water was changed every two days to prevent the built up of a film of scum that may potentially suffocate the larvae. The pupae were pipetted and separated from larvae onto another container to await eclosion. The adults that eclosed were fed with 10% sugar solution through a piece of soaked cotton wool placed over the top of net. The rearing was done at room temperature ( $25 \pm 1^\circ\text{C}$ ; RH  $65 \pm 10\%$ ; 12 L: 12 D photoperiod).

### 3.1.3 Adult mosquito identification

Upon eclosion, adult mosquitoes were identified (as *An. gambiae* sibling species) using gross morphology (Gillies and Coetzee, 1987) by employing the microscope (Model: BS-3060BT, BestScope International Ltd. Beijing, China). The TaqMan assay was used in the blind genotyping trial to confirm *An. arabiensis* from sibling species: *An. gambiae* s.s. and *An. quadriannulatus*. PCR reactions (24 µl) contained in a PCR tube with a mosquito leg covered with the mastermix: 12.5 µl of SensiMix DNA kit (Quantace), 800 nM of each primer and 200 nM of each probe. Samples were run on 7500 Fast Real-Time PCR (Applied Biosystems®) using the temperature cycling conditions of: 10 minutes at 95°C followed by 45 cycles of 95°C for 15 seconds, 50°C for 20 seconds and 72°C for 20 seconds. The increase in VIC and FAM fluorescence was monitored in real time by acquiring each cycle on the yellow (530 nm excitation and 555 nm emission) and green channel (470 nm excitation and 510 emission) respectively (Bass *et al.*, 2007).

### 3.1.4 Insecticide Susceptibility Bioassays

The insecticide susceptibility bioassays (available in kit form supplied by Universiti Sains Malaysia (USM), Penang, Malaysia,) were done as per standard World Health Organization (WHO) procedure (WHO, 2016). Female mosquitoes (non blood-fed) that were 2 to 5 days old were fed (10% sugar solution) in rearing cage (BugDorm- 43030F, 240mm<sup>3</sup>, Megaview Science Co., Ltd, Taiwan, China) ready for the bioassays. The mosquitoes used were randomized across treatments so that the differential mass may not have a confounding effect on the results. The principle behind the bioassays (time mortality bioassays) was that mosquitoes were exposed (by contact) to surfaces impregnated by a discriminating insecticide doses over a period of time (WHO, 1998). The susceptibility tests were done with four common classes of recommended insecticides; organophosphates (malathion 5%), organochlorines (Dichloro-Diphenyl-Trichloroethane [DDT]) 4.0% and dieldrin 0.4%, dieldrin 4%, carbamates (propraxur 0.1%, bendiocarb 0.1%) and pyrethroids (lambda-cyhalothrin 0.05%, permethrin 0.75%, deltamethrin 0.5%, cyfluthrin 0.15% and etofenprox 0.5%). A batch of 20 mosquitoes were transferred (by aspirators) from rearing cages into a total of 5 holding tubes, four of which (green dotted) were replications to be exposed to insecticides while the other was used for the control. Another set of exposure tubes were made available, four lined with impregnated insecticide (red dotted) and a control tube was lined with a paper with oils that lined the insecticide impregnated papers. The controls were lined with specific oils for organochlorines (risella oils), pyrethroids

(silicone oils) and both organophosphates and carbamates lined with olive oil. The holding tubes and the exposure tubes were fastened together by a slide (Fig. 2A) after which, it was opened and mosquitoes were gently blown to the exposure tubes. Mosquitoes that got damage during the transfer were replaced before the exposure period. After a successful transfer, the holding tubes were removed leaving the exposure tubes upside down on the slides. For every 15 minutes (up to 60 minutes), the knockdown mortalities were observed and recorded. At the elapse of 1 hour, the mosquitoes were transferred back to the holding tubes then kept for the next 24 hours with a cotton wool (soaked in 10% sugar solution) placed on top (Fig. 2B). Mosquitoes that survive 24 hours post this treatment may have developed resistance (MoH, 2016). Surviving mosquitoes (post 24 hour pesticide treatment) were killed (by putting in a -20°C freezer for 10 minutes) then placed in a 2 mL eppendorf tube with a desiccating silica gel (Glassworld: Orange particle size- 2,8 to 7.0mm, South Africa) below a small piece of cotton wool for further processing. Those that died, were kept in batches of 20 in a 25 mL perforated vials then put in a zip lock bag containing 50g of silica gel for further molecular identification.

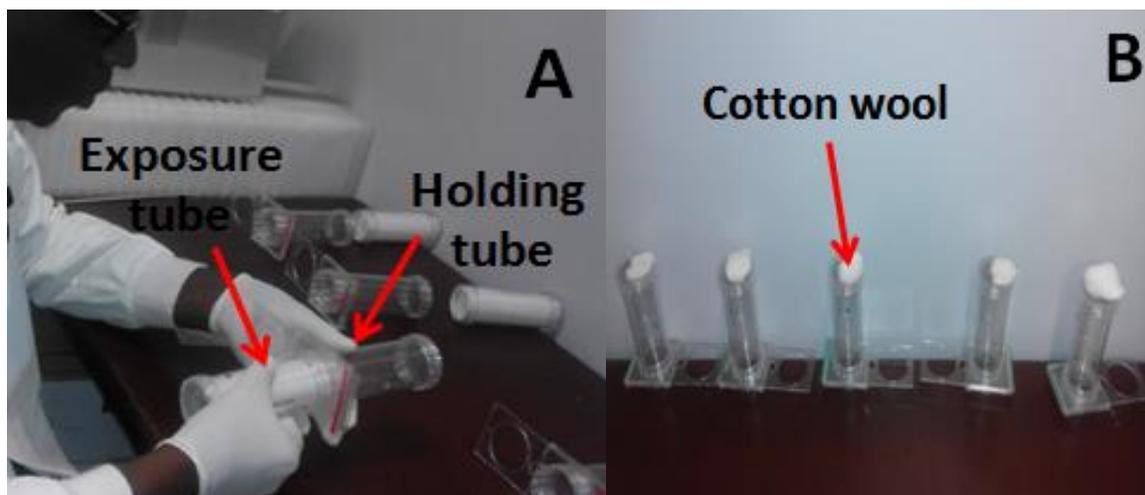


Figure 2: Exposure tube (red dotted) and holding tube (green dotted) held together by a slide during mosquito transfer (A), Mosquitoes in holding tubes with cotton wool (soaked in 10% sugar solution) for 24 hours post exposure (B).

### 3.1.5 Data analysis

Mosquito mortality for insecticide susceptibility testing was analyzed as percentage using Abbott (1987) formula for control, exposure and corrected exposure mortalities (Fig. 3). Furthermore, for this analysis, the corrected exposure mortality formula was ignored if control mortality was below

5%, but was used only when the control mortality was between 5% and 20%. However, if control mortality was more than 20%, the experiment was discarded. Overall, mosquitoes efficacy to tested insecticides were defined as susceptible ( $\geq 98\%$  mortality), suspected resistance (90- 97% mortality) and resistant ( $< 90\%$  mortality) (WHO, 2016).

$$\text{Control Mortality (C)} = \frac{\text{No. of dead mosquitoes}}{\text{Total No. of mosquitoes in control tubes}} \times 100$$

$$\text{Exposure Mortality (E)} = \frac{\text{No. of dead mosquitoes}}{\text{Total No. of mosquitoes in the exposure tubes}} \times 100$$

$$\text{Corrected Exposure Mortality (CEM)} = \frac{E - C}{100 - C} \times 100$$

Figure 3: Formulae for calculating control mortality (C), exposure mortality (E) and corrected exposure mortality (CEM) percentages (Adapted from Abbott, 1987).

The knockdown time 50% (KDT<sub>50</sub>) of total tested female *An. arabiensis* mosquitoes for each insecticide were pooled together and subjected to probit analysis from the Statistical Package for the Social Sciences (SPSS) software (Version 24). Prior to this, the percentage knockdown values obtained (averages of four replicates) were calculated using the formula by Ramar *et al.* (2014) (Fig. 4).

$$\text{Knockdown (\%)} = \frac{\text{No. of adults Knocked- down (per unit time)}}{\text{No. of adults tested}} \times 100$$

Figure 4: Formula for calculating knockdown percentage. (Adapted from Ramar *et al.*, 2014).

## 3.2 Results

### 3.2.1 Insecticide susceptibility

The study sites (districts) generally reported insecticide susceptibility (>98% mortality) to malathion 5%, propoxur 0.1%, bendiocarb 0.1%, DDT 4.0% with susceptibility on Dieldrin 4% , Cyfluthrin 0.15% and Etofenprox 0.5% exclusive for Bobirwa district (Table 1). Reduced sensitivity of mosquitoes to insecticides was generally noted for organochlorines and the pyrethroids (Table 1). Mosquitoes from Okavango showed resistance (<90% mortality) to pyrethroids; lamda- cyhalothrin 0.05%, permethrin 0.75%, deltamethrin 0.5% and cyfluthrin 0.15% (78.8%, 78.8%, 81.3% and 83.8% mortality respectively). Resistant to lamda-cyhalothrin 0.05% and permethrin 0.75% was observed only from Ngamiland mosquitoes (Table 1). Though Bobirwa mosquitoes did not report resistance to insecticides, suspected resistance (90- 97% mortality) was observed with the organochlorines (dieldrin 0.4%) and the pyrethroids (lamda-cyhalothrin 0.05%, permethrin 0.75% and deltamethrin 0.5%) (Table 1). Overall, lamda-cyhalothrin 0.05% and permethrin 0.75% recorded resistance on mosquitoes from all the districts. Moreover, it was noted that amongst other districts, mosquitoes from Okavango showed prominent resistance to pyrethroids tested. The two dosages of dieldrin (0.4 and 4%) generally yielded suspected resistance across all the study sites.

Table 1: A summary of percentage mortality 24 hours after a 1- hour exposure to different classes of insecticides on field collected F1 progeny of *An. arabiensis* from Okavango, Ngamiland and Bobirwa districts.

Class	Insecticide Name	District and Resistance status		
		Okavango (n=1100)	Ngamiland (n=1100)	Bobirwa (n=1100)
<b>Organophosphates</b>	Malathion (5%)	100 (S)	98.8 (S)	100 (S)
<b>Organochlorines</b>	DDT (4.0%)	96 (SR)	97.5 (SR)	98.8 (S)
	Dieldrin (0.4%)	93 (SR)	95 (SR)	97.5 (SR)
	Dieldrin (4%)	95 (SR)	96.3 (SR)	98.8 (S)
<b>Carbamates</b>	Propoxur (0.1%)	98.8 (S)	100 (S)	100 (S)
	Bendiocarb (0.1%)	100 (S)	100 (S)	98.8 (S)
<b>Pyrethroids</b>	Lamda-cyhalothrin (0.05%)	<b>78.8 (R)</b>	<b>81.3 (R)</b>	95 (SR)
	Permethrin (0.75%)	<b>78.8 (R)</b>	<b>83.8 (R)</b>	95 (SR)
	Deltamethrin (0.5%)	<b>81.3 (R)</b>	92.5 (SR)	96.3 (SR)
	Cyfluthrin (0.15%)	<b>83.8 (R)</b>	97.5 (SR)	98.8 (S)
	Etofenprox (0.5%)	97.5 (SR)	96.3 (SR)	98.8 (S)

Letters in the parentheses indicated resistance status of tested mosquitoes (S: susceptible, SR: suspected resistance and R: resistant). All pesticides indicated in bold symbolize cases of suspected confirmed resistance (<90% mortality).

### 3.2.2 Knockdown time (KDT<sub>50</sub>)

The pyrethroids (lamda-cyhalothrin 0.05%, permethrin 0.75%, deltamethrin 0.5%, Cyfluthrin 0.15% and Etofenprox 0.5%) generally reported the lowest KDT<sub>50</sub> as compared to other classes of insecticides with deltamethrin 0.5% scoring the lowest value of 17.281 minutes for Bobirwa mosquitoes (Table 2). In contrast, the carbamates propoxur 0.1% and bendiocarb 0.1%) generally scored the highest KDT<sub>50</sub> of the tested mosquitoes, with Propoxur 0.1% having the highest value of 47.994 minutes for Okavango mosquitoes (Table 2). However dieldrin 0.4% was not different from the carbamates.

Table 2: A summary table of mean knockdown time (KDT<sub>50</sub>) (minutes) of *An. arabiensis* F1 progeny (n= 100) from malaria endemic districts tested against different classes of insecticides with ±95% confidence intervals (CI).

Insecticide		Mean knockdown time KDT <sub>50</sub> (Min) ±95% CI.		
Class	Name	Okavango	Ngamiland	Bobirwa
<b>Organophosphates</b>	Malathion (5%)	39.073	39.294	38.352
<b>Organochlorines</b>	DDT (4.0%)	28.574	25.721	31.229
	Dieldrin (0.4%)	47.613	46.365	46.652
	Dieldrin (4%)	45.86	39.558	32.753
<b>Carbamates</b>	Propoxur (0.1%)	47.994	47.284	46.626
	Bendiocarb (0.1%)	40.636	46.526	46.700
<b>Pyrethroids</b>	Lamda-cyhalothrin (0.05%)	23.01	18.541 (0.288- 27.807)	23.559 (0.058- 27.194)
	Permethrin (0.75%)	18.707	21.901	20.85
	Deltamethrin (0.5%)	17.859	19.195 (0- 25.864)	17.281
	Cyfluthrin (0.15%)	25.798	32.794	38.603
	Etofenprox (0.5%)	28.779	35.24	39.137

### 3.3 Discussion

#### 3.3.1 Insecticide susceptibility

The evolution of insecticide resistance in malaria vectors is currently the main cause of morbidity and mortality in sub-Saharan Africa (Weetman and Donnelly, 2015). Though burdening and threatening to the livelihood, some reports have demonstrated a decrease in malaria transmission due to massive roll outs of the long lasting insecticide-treated bed nets (LLINs) in Africa (Thomas and Read, 2016). In Botswana, a combination of vector control strategy using LLINs and indoor residual spraying (IRS) has been practiced continuously over the years (MoH, 2012). However, Okumu and Moore (2011) suggested that the two intervention strategies may not be complimentary. Rather, they may exacerbate the evolution of insecticide resistance in mosquito populations due to increased pesticide selection pressure on indoor 'unavailable' mosquito resting places (Yebakima *et al.*, 2004). Despite the pesticide persistence and environmental toxicity status of DDT and other WHO recommended and locally registered insecticides (Vieira *et al.*, 2001), Botswana has been able to effectively reduce malaria cases over the years through chemical based intervention strategies (Simon *et al.*, 2013). Parallel to this achievement, this study demonstrated vector responses on continuous exposure to insecticides may be negative and increases vectors' capacity to become less sensitive to insecticides used for IRS and LLINs (Dhiman *et al.*, 2016). The reduced mosquito sensitivity to insecticides observed in this study may be due to the prolonged (>60 years) use of IRS (DDT and lambda-cyhalothrin) and distribution of LLINs (pyrethroid impregnated) (see WHO, 2012; Simon *et al.*, 2013; Chihanga *et al.*, 2016). Moreover, lambda-cyhalothrin (pyrethroid) and DDT (organochlorine) are regarded as related classes of insecticides with similar mode of action (Coats, 1990; Sanchez-Bayo, 2012). Therefore, it may be to this generalization that mosquitoes from the study sites were already insensitive to pyrethroids (permethrin, deltamethrin, cyfluthrin and etofenprox) not currently in use for vector control locally. Furthermore, this was the case with lambda-cyhalothrin and permethrin (Table 1) which reported resistance across districts though permethrin has no prior record of use in the districts that may be linked to their associated mode of action. Okavango district showed prominent insecticide resistance on *An. arabiensis* mosquitoes which may be associated with selection pressure from pesticidal usage from the households in the area. Thus, Okavango district generally attained a high yearly (2008 to 2012) mass distribution of LLINs as compared to Ngamiland and Bobirwa (see Simon *et al.*, 2013). Indeed, Bobirwa was less prominent on insecticide resistance, and may be

associated with fewer acceptances of national intervention strategies (eg. LLINs) as elucidated by Simon *et al.* (2013) thus ‘delaying’ resistance on *An. arabiensis* for the district.

Though not conclusive, *An. arabiensis* as one of the important malaria vectors in the country (Tawe *et al.*, 2017), may be showing a genetic drift towards resistance as reported in other Southern African neighboring countries (see Munhenga *et al.*, 2008; Nardini *et al.*, 2013). The current study adds to other reports on insecticide resistance in time and space, and may be extended to other malaria vectors on fine scale (Matowo *et al.*, 2017) and large scale vector- responses to insecticides for both the endemic and non-endemic districts of the country (Padonou *et al.*, 2012). With the resistance reported here, the country should integrate the current national intervention strategies with other approaches of vector management in minimizing resistance and simultaneously getting environmental benefits. This may include complimentary methods such as eliminating unnecessary permanent and/or temporary breeding places or the use of non- chemical larvicide (eg. *Bacillus thuringiensis israelensis* or plant extracts) in such stagnant pools for the reduction of the larval stage of the *Anopheles* mosquitoes (Ghosh *et al.*, 2012; Dambach *et al.*, 2014). In areas such as Okavango delta where persistence chemical usage may threaten the natural world heritage biodiversity (Ramberg *et al.*, 2007), the country may need to be more conservative on the use of pyrethroids, thus, sustainable alternatives of species specific soft and botanical pesticides may be recommended as they are environmental friendly (Sarwar and Salman, 2015). In addition, larvicide using plant extracts may be recommended in infested swampy areas, natural ponds and lagoons that make up the Okavango delta (see Ghosh *et al.*, 2012). Moreover, those in rural areas of the Okavango and elsewhere, who may opt not to use artificial chemical based repellants, may instead use the anti- mosquito plants and their derivatives as natural deterrents within their households (De Boer *et al.*, 2010; Innocent *et al.*, 2014).

In addition, the use of biological control methods may be used in dams or large artificial water bodies using predators such as fish, shrimps, dragon flies to feed on the pupal and larval stages of the *Anopheles* mosquitoes (Chandra *et al.*, 2008; Faithpraise *et al.*, 2014; Coelho *et al.*, 2017). Furthermore, innovative methods that target mosquitoes at different points (eg. oviposition, sugar or blood feeding, mating) may be used as novel complimentary approaches in vector control in the country as a way of minimizing the use of insecticides which may be linked to the current resistance (Gantz *et al.*, 2015; Muema *et al.*, 2017; Willyard, 2017). For example, the sterile insect techniques may potentially be used to suppress the virulence of the wild female *An. arabiensis* population thus reducing parasite transmission (Munhenga *et al.*, 2011; Lees *et al.*, 2015; Dandolo *et al.*, 2017).

Furthermore, the spread of *Wolbachia* bacteria through mosquito populations has recently become a promising strategy to modify vector species populations due to a sperm–egg incompatibility (cytoplasmic incompatibility) (Caragata *et al.*, 2016; Shaw *et al.*, 2016; Jiggins, 2017) This may ultimately add to the integrated vector management (IVM) approach that aims to drive a framework for decision making, continued insecticide resistance monitoring and evaluation, stake-holder involvement, and the implementation of insecticide resistance management strategies (Chanda *et al.*, 2017). Indeed the IVM for managing insecticide resistance is a priority for Okavango where insecticide resistance was evident for the pyrethroids (Table 1) and its highest malaria cases amongst endemic districts and non-endemic districts (Chihanga *et al.*, 2016).

The mosquitoes assessed were entirely susceptible to the carbamates and the organophosphates (malathion, propoxur and bendiocarb). Firstly, this may be because the tested mosquitoes populations did not have prior exposure to these insecticides and hence more susceptible. Although mosquitoes' mass were not recorded for this study, mosquitoes were randomized in all treatments since mass affect susceptibility to insecticides (Alshawish *et al.*, 2004; LaVerda *et al.*, 2015). Second, susceptibility may be accounted for by differential mechanisms between carbamates and organochlorines and pyrethroids. The carbamates and the organophosphates are aimed to inhibit the normal breakdown of Acetylcholine (ACh) which is a synaptic neurotransmitter that transmits signals from one neuron to another (Fukuto, 1990). The inhibited activity of Acetylcholinesterase (enzyme) on ACh, may result in over-stimulation of the neurons due to accumulation of ACh thus leading to insect paralysis and subsequent death (Corbel *et al.*, 2009). This mode of action is in contrast with that of organochlorines (DDT) and pyrethroids where the voltage-gated sodium channel proteins found in insect nerve cell membranes are a target (Davies *et al.*, 2007). The country's national vector control strategies are based on insecticides that target one site (voltage- gated sodium channel proteins) which may exert a selection pressure for possible mutation. Therefore it may be logical, from the perspective above to use insecticides with different mode of action on rotational/alternation to improve efficacy while simultaneously managing insecticide resistance (Sparks and Nauen, 2015).

Overall, this work provides critical information to malaria vector control programmes on mosquito insecticide susceptibility status. The insecticides that were efficacious from organophosphates and carbamates (malathion, propoxur and bendiocarb) may be used as alternatives in the country. This may reduce insecticide selection pressure on DDT and pyrethroid currently used (lambda-cyhalothrin) with possible reduced outcome chances of resistance. Therefore, this work

suggests alternation of pesticidal use (Tangena *et al.*, 2013) for vector control especially with mosquitocidal carbamates though longer duration of action but generally lower in toxicity to other insects and the environment (Swale *et al.*, 2015). Although the national vector control strategy is based on the continuous use of DDT and pyrethroids (eg. lamda- cyhalothrin for IRS), there is need for future continuous monitoring and evaluation of their impact on vector response to avoid resistance. Finally, there is need for future molecular confirmation of possible insecticide resistance (including cross resistance) in malaria vectors and their mechanisms for appropriate control strategies towards malaria elimination in the country.

### 3.3.2 Knockdown time (KDT<sub>50</sub>)

Assessment and selection of insecticides based on their time of action for vector control is an essential component that has a bearing in future cases of vector disease prevalence. The selection may be based on policies in place, environmental consideration, health implications, resistance status of the controlled population and the efficacy of the insecticide (Zilberman and Millock, 1997; Damalas and Eleftherohorinos, 2011; Andersson *et al.*, 2014; Smirle *et al.*, 2017). With the above assessment criteria in mind, the time taken to knockdown 50% of the vector population by a discriminatory dose may be useful in selection of an appropriate insecticide for vector control. Results of this work may assist on information surrounding insecticide selection for overall management of insecticide resistance on malaria vectors. The pyrethroids generally reported shorter KDT<sub>50</sub> than other classes tested. This is in keeping with (Wakeling *et al.*, 2012) and supports the notion this group of insecticide is fast in action. Iregardless of this fast in activity, this group appeared not to be efficacious for *An. arabiensis* at least in Okavango and Ngamiland, indicating potential vector pyrethroid resistance. In contrast, malathion, propoxur and bendiocarb were generally observed to be slow to action (high KDT<sub>50</sub>) across the study sites though mosquitoes reported susceptible their discriminating dozes. Overall, most fast-acting insecticides inhibit the transmission of nerve impulses while slower acting insecticides inhibit specific enzymes for activity, block the electron chain transport or disrupt the hormonal action in the insect body (Sparks and Nauen, 2015). The ultimate result may be death of mosquitoes post exposure to insecticide toxicity (whether fast or slow-acting) depending on the resistance status of the mosquitoes tested (see Brooke *et al.*, 2015; Rakotoson *et al.*, 2017). Therefore, it may follow that, if mosquitoes do not show resistance, a fast-acting insecticide may be given priority of choice but with pyrethroid-resistance in the tested *An. arabiensis* (as in Okavango), slow-acting

insecticides may serve as alternatives. Hence, it may be important that vector response assessment on both the KDT<sub>50</sub> and insecticide susceptibility status be carefully considered and merged appropriately for future insecticide selection for managing resistance.

### 3.4 Conclusion

Though pyrethroids were observed to be fast in action, *An. arabiensis* displayed a notable reduction in sensitivity which has implications on future vector control strategies using this insecticide group. This baseline assessment work suggests for continuous monitoring of insecticide resistance to all potential malaria vectors in the country before conclusive recommendations on susceptibility status are made. Indeed, the current research affirms insecticide resistance is currently challenging and a burden in vector control and associated disease management (Balkew *et al.*, 2012). Thus, malaria elimination in the country is a priority that necessitates efforts in vector management to monitor insecticide resistance. This may be achieved using integrated approaches that complement the current vector management strategies in minimizing resistance and at the same time delivering environmental benefits. For example, the sterile insect techniques, botanical pesticides, biological control (eg. *Wolbachia* bacteria) and other novel mosquito transgenic technologies that may be applicable for vector species in the country and other malaria endemic African countries (Bourtzis, 2008; Alphey *et al.*, 2010; George *et al.*, 2014).

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## **CHAPTER 4**

**Thermal biology of wild *Anopheles arabiensis* (Diptera: Culicidae):  
implications on vector responses to climate change and population  
dynamics.**

#### 4.0 Introduction

Anthropogenic climate change represents a significant societal challenge, and impacts on the growth, survival and the future of biodiversity (Bellard *et al.*, 2012; Aukema *et al.*, 2017). Insects, as sensitive ectotherms, need to survive and adapt to daily and seasonal climate fluctuations in their habitats for their overall fitness (Paaijmans *et al.*, 2013). Indeed, they need to cope with such variability in the short-, medium- to long term through physiological adjustments. One way in which insects are able to mitigate thermal stress, is through phenotypic plasticity, which is the phenotypic malleability of an insect in response to changes experienced in environmental conditions (Chown and Nicolson, 2004). Though the mechanism is short term, it may also be long term, where by insects may subsequently adapt (through physiological responses) to changing climatic variations in new environments as a way of avoiding extinction and establishing populations (Chown *et al.*, 2007; Nyamukondiwa *et al.*, 2010; Merilä and Hendry 2014; Valladares *et al.*, 2014). Through short term plasticity (rapid cold hardening), ectotherms can adjust and suffer less from exposure to low temperatures on a short timescale (Powell and Bale, 2005) whereas, developmental acclimation (or acclimatization in the field) are long term phenotypic plastic responses that adapt insects over moderate to long time scales (Gerken *et al.*, 2015). Physiological traits (over long period of time) may also develop through mechanisms of natural selection and evolution (Lee *et al.*, 2007) and other behavioral thermoregulation activities and mechanisms (Angilletta, 2013). Adaptation to climate variability is not only limited to impacting insects thermal tolerance but also affects their overall population dynamics and distribution. Indeed, according to Bai *et al.* (2013), variability in temperature, precipitation, wind, and extreme weather events are linked to transmission of mosquito-borne diseases and their densities. To date, research has shown increasing temperatures may facilitate progressive extension of malaria, through extension of the associated vectors (Aal and Elshayeb, 2011). Mosquito vectors are constantly exposed to seasonal changes of climate variability thus impacting on their ‘numbers’ and their ability to transmit pathogens. Furthermore, increased temperature is a driver of essential mosquito and parasite life history traits that determine rapid parasite development and survival and an increased transmission risk (Blanford *et al.*, 2013; Mordecai *et al.*, 2013). The seasonal cycles of low (winter) and optimum (summer) temperatures have established mosquito physiological traits of arresting activity (diapause/ quiescence) and active behavioral responses that enhance growth, reproduction and pathogen transmission during ‘peak malaria seasons.’

Measures of thermal tolerance have been used and appreciated over the years, to investigate the thermal capacity of insects in their holocoenotic environments (Bowler and Terblanche, 2008; Chidawanyika *et al.*, 2017). As such, critical thermal limits (CTLs) have been especially useful in providing ecologically relevant species thermal functional limits and thus thermal windows for key life history activities (Terblanche *et al.*, 2007). The CTLs are therefore described by the critical thermal maximum ( $CT_{max}$ ) and the critical thermal minimum ( $CT_{min}$ ). Critical thermal minimum represents the lowest temperature allowing insect activity, and at  $CT_{min}$ , insects lose the fitness ability to respond or coordinated muscle stimuli (Block *et al.*, 1990). Critical thermal maximum is the highest temperature allowing insect activity, and represents a point where locomotory activity becomes disorganized, characterized by onset of muscle spasms, leading to physiological failure and death (Lutterschmidt and Hutchison, 1997; Chown and Nicolson, 2004). Thus both traits represent ecologically relevant measures of fitness traits, beyond which key life sustaining processes are compromised. In the current imminent climate change, it therefore remains a need to understand mosquito vector CTLs and explore their ecological relevance in mosquito activity (in response to temperature) such as, but not limited to, its behavior (feeding, biting, flying, mating), population modeling and forecast with implications on vector fitness and associated diseases. The imminent extreme climatic conditions on vector-borne disease outbreak patterns (Anyamba *et al.*, 2014) and the effect of recurring natural disasters in certain parts of the country, for example floods (see e.g. Thakadu *et al.*, 2017) make it interesting to explore malaria mosquito vector thermal functional limits to activity as a measure of performance to extreme temperatures with implications on planning and management strategies for future vector-borne disease transmission (Lyons *et al.*, 2012).

Currently, there is lack of information on physiological responses of mosquito life stages to temperature. Nevertheless, the information remains critical in mapping the activity of these vectors upon introduction to new areas, and the associated disease dynamics. The objectives of this chapter were therefore to (i) determine the CTLs of *An. arabiensis* mosquitoes and (ii) investigate the effects of sex and developmental stage (adult versus larva) on thermal tolerance. Such traits will help indicate functional thermal windows for the vector, and may help explain, whether the vector may thrive upon introduction into new areas.

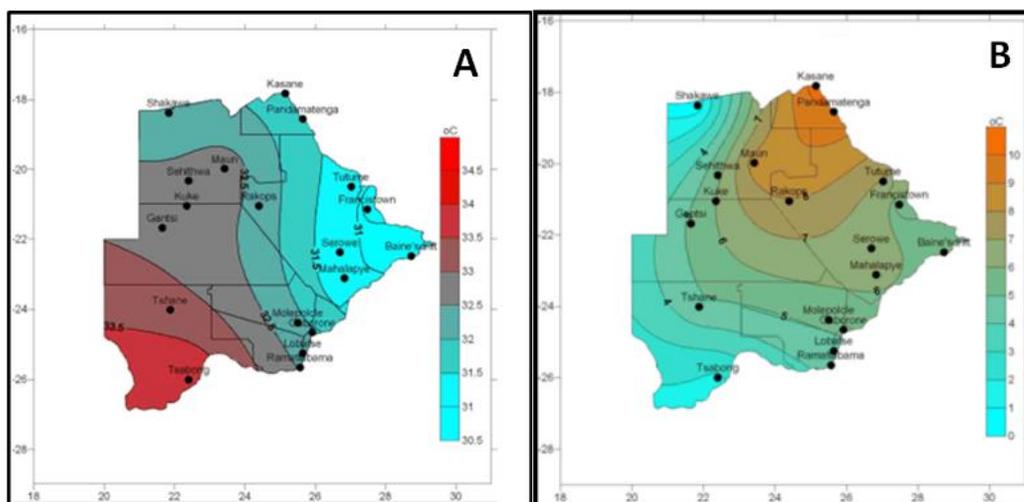
## 4.1 Materials and Methods

### 4.1.1 Larval sampling

The *Anopheles* larvae were collected from stagnant pools (Bobirwa: S22. 17166°, E028. 91298°, Ngamiland: S20. 01830°, E023. 24501° and Okavango: S18, 17043°, E021, 50967°) in the summer season 2015/ 2016 and 2016/ 2017 on February to March. The average monthly maximum and minimum temperatures and average annual rainfall data for Bobirwa, Ngamiland and Okavango were obtained from the meteorological services for the July 2015 to June 2017 period (Table 1; Fig. 1). The larval sampling consent was obtained from the Ministry of Health (Permit: PPME 13/18/1 PS V (353) and community leaders from sampled areas. The collection was done from morning until noon (0800hrs to 1200hrs) during a clear daylight for effective visualisation of the larvae on the water surfaces. When approaching water sources, precautions were taken not to disturb the larvae and the shadow was always away from the water source as it disturbed the larvae. The collection was done with a larval scoop and a larval tray then transported in netted containers kept in cooler boxes to the laboratory for further processing.

Table 1: Average monthly maximum and minimum temperatures and average annual rainfall for July 2015 to June 2017 from Bobirwa, Ngamiland and Okavango districts.

	Bobirwa	Ngamiland	Okavango
Average monthly minimum (°C)	14.6	17.2	14.5
Average monthly maximum (°C)	29.4	31.1	31.9
Average annual rainfall (mm)	351.1	516	394.9



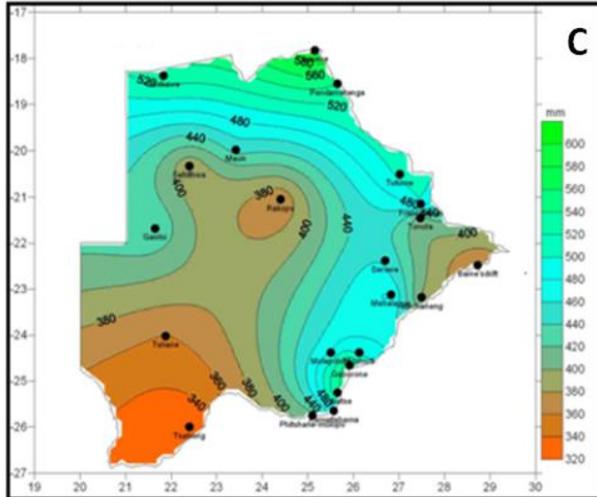


Figure 1: Map of Botswana showing average monthly maximum temperatures (A), average monthly minimum temperatures (B) and average annual rainfall distribution patterns (C). Adapted from Mutamiswa *et al.* (2017).

#### 4.1.2 Rearing to adult mosquitoes

The mosquitoes larvae collected from water sources (in different districts) were sieved carefully through a strainer and placed onto another container with clear river water from the sampled sites in maintaining their natural habitat *in situ*. The container was covered with a net that allowed gaseous exchange for effective aeration on the mosquito life stages. The larvae were fed with a scoop of fish food (Sera: Vipar family) daily and the water was changed every two days to prevent the built up of a film of scum that may potentially suffocate the larvae. The pupae were pipetted and separated from larvae onto another container to await eclosion. The adults that eclosed were fed with 10% sugar solution through a piece of soaked cotton wool placed over the top of net. The rearing was done at room temperature ( $25 \pm 1^\circ\text{C}$ ; RH  $65 \pm 10\%$ ; 12 L: 12 D photoperiod).

#### 4.1.3 Adult and larval mosquito identification

The *Anopheles* mosquito larvae in stagnant waters (prior to collection) were firstly identified by their common resting behavior (parallel to water surfaces). Then later (after collection) placed in a depression microscopic slide with a minimum amount of water and identified under a light microscope

(objective x10) using morphological keys by Gunathilaka *et al.* (2014). The 4th instar larvae were identified and used for the experiments since were considered physiologically mature.

Upon eclosion, adult mosquitoes were identified using gross morphology (Gillies and Coetzee, 1987) as *An. gambiae* siblings. The TaqMan assay was used in the blind genotyping trial to confirm *An. arabiensis* from sibling species: *An. gambiae* s.s. and *An. quadriannulatus*. PCR reactions (24  $\mu$ l) contained in a PCR tube with a mosquito leg covered with the mastermix: 12.5  $\mu$ l of SensiMix DNA kit (Quantace), 800 nM of each primer and 200 nM of each probe. Samples were run on 7500 Fast Real-Time PCR (Applied Biosystems®) using the temperature cycling conditions of: 10 minutes at 95°C followed by 45 cycles of 95°C for 15 seconds, 50°C for 20 seconds and 72°C for 20 seconds. The increase in VIC and FAM fluorescence was monitored in real time by acquiring each cycle on the yellow (530 nm excitation and 555 nm emission) and green channel (470 nm excitation and 510 emission) respectively (Bass *et al.*, 2007).

#### **4.1.4 Critical thermal limits (CTLs)**

Adults (2-5 days old) and the larvae (4th instar) were used for CTLs assays. The mosquitoes were transferred using aspirators from rearing containers separated by sex. Each mosquito was weighed using a microbalance (Model: AS 220.R2, RADWAG wagl Elektroniczne, Poland) before subjected to experiments. Firstly, the eppendorf tubes were labeled and separately weighed then secondly, mosquitoes were transferred into the tubes for weighing. Therefore, the mass (in grams) of an individual mosquito was the difference between the mass of the eppendorf tube and the eppendorf tube with the mosquito. Ten mosquitoes were used as replicates and this was repeated twice to give n=20.

Critical thermal limits were measured using a programmable waterbath (Lauda Eco Gold, Lauda DR.R. Wobser GMBH and Co. KG, Germany) that provided ramping rates and was attached to the double jacketed chamber, which had 11 organ pipes. The ecologically relevant ramping rate (0.25°C.min<sup>-1</sup>) was used for measuring the CTLs (Terblanche *et al.*, 2011), ramping from 25°C up (for the CT<sub>max</sub>) and down (CT<sub>min</sub>) until CTLs. One pipe (middle) had a thermocouple (type K 36 SWG) connected to a digital thermometer (53/54IIB, Fluke Cooperation, USA). The tube with a thermocouple was used in providing the temperature experienced by the adult mosquitoes in the rest of the tubes thus representing the ambient temperature (see protocols of Nyamukondiwa and Terblanche, 2010). For small ectothermic insects, it has been shown that their body temperature closely

approximate ambient temperatures (see Stevenson 1985). The liquid that flowed through the double jacketed chamber was water and propylene glycol (ratio 1:1). This mixture of the two prevented the freezing of the liquid at sub- zero temperatures. Mosquitoes were transferred into the organ pipes and as temperature was ramping, they were gently probed with a thermally inert bush to assess coordinated responses to muscle stimuli, failure of which defined the  $CT_{max}$  or  $CT_{min}$ . As for the larvae, about 5mL of river water (from native environment) was added into organ pipes (before being transferred), one of which had likewise, a thermocouple that recorded the water temperature experienced by the larvae. Critical thermal limits for larvae were defined as inability for coordinated muscle response following gentle prodding, as in the adults.

#### 4.1.5 Data analysis

Data on CTLs variances was checked normality and homogeneity using Shapiro-Wilk's tests and Levene's tests, respectively and satisfied the assumptions of ANOVA. The data was therefore analysed using both factorial ANOVA (adults) and one way ANOVA (larvae) in STATISTICA (Version 13.2, Statsoft Inc., Tulsa, Oklahoma, USA). Turkey Kramer's posthoc tests were used to separate statistically heterogeneous groups. Data on mosquito mass was analysed with factorial ANOVA to see the effect on CTLs. However it was not a significant factor on the CTLs hence it was omitted.

## 4.2 Results

### 4.2.1 Critical thermal limits for adults

Sex and site (district) of mosquito collection had significant effects on mosquito  $CT_{min}$  (Fig. 2A; Table 2). Adult female *An. arabiensis* had a significantly enhanced  $CT_{min}$  than males (Fig. 2A). Furthermore, mosquitoes from Okavango appeared to have a significantly enhanced  $CT_{min}$  relative to the other two sites, with Bobirwa having the worst (Fig. 2A). The district x sex interaction was however not significant on the  $CT_{min}$  (Fig. 2A; Table 2). Similarly, sex and site (district) of mosquito collection had significant effects on  $CT_{max}$  (Fig. 2B; Table 2). Adult female *An. arabiensis* had a significantly enhanced  $CT_{max}$  than males (Fig. 2B). Moreover, mosquitoes from Bobirwa had significantly reduced  $CT_{max}$  compared to those from Ngamiland and Okavango (Fig. 2B). The district x sex interaction was not significant on the  $CT_{max}$  (Fig. 2B; Table 2).

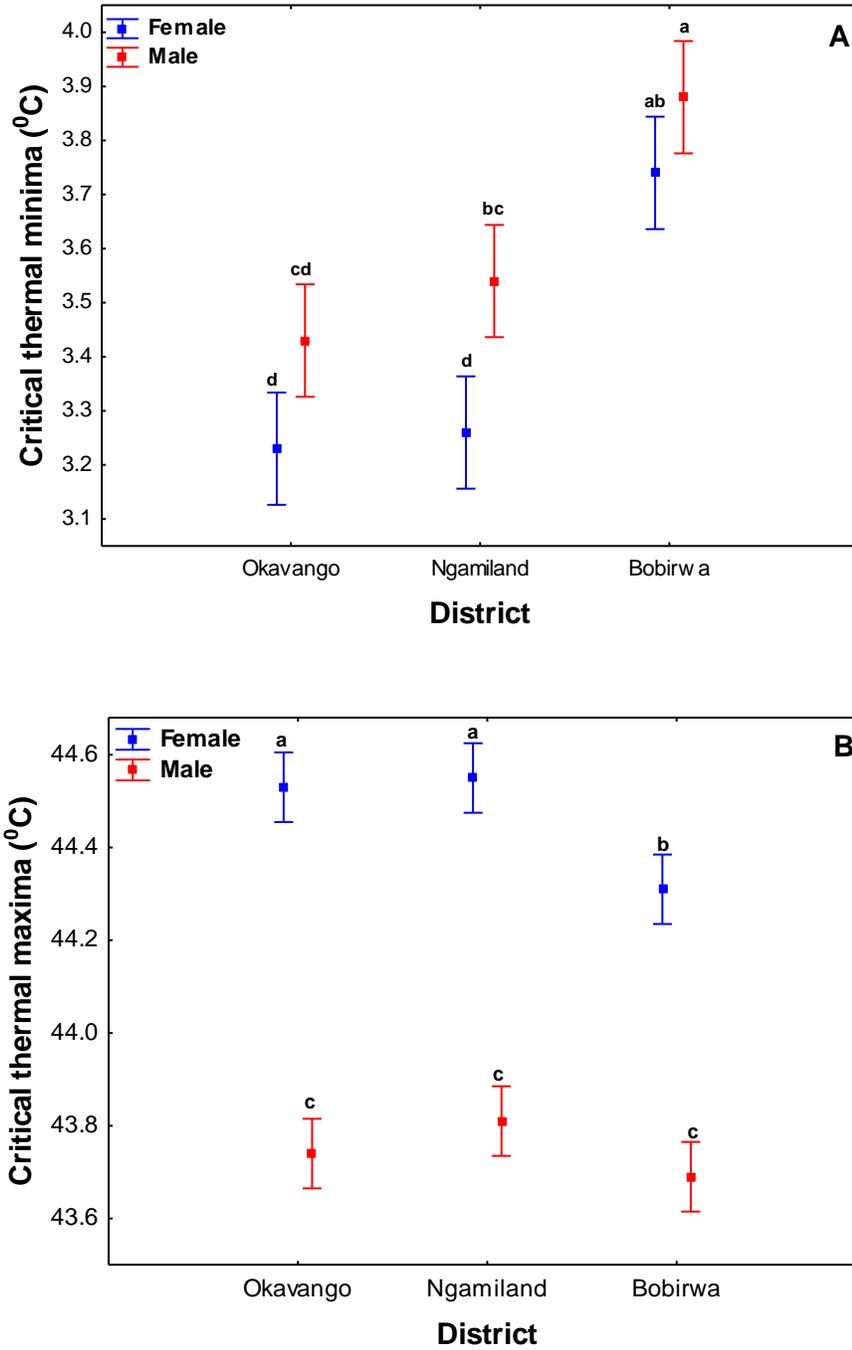


Figure 2: Adult *An. arabiensis* (n=20) male and female Critical thermal minima (A) and critical thermal maxima (B). Each point represents mean  $\pm$ 95% CL. Means with the same letter are not statistically different.

Table 2: A summary of factorial ANOVA on *An. arabiensis* mosquito critical thermal minima and critical thermal maxima.

<b>Trait</b>	<b>Effect</b>	<b>F- value</b>	<b>P- value</b>
<b>CT<sub>min</sub></b>	Sex	F(1,54)= 23.9	< <b>0.0001</b>
	District	F(2,54)= 50.1	< <b>0.0001</b>
	District x Sex	F(2,54)= 0.92	=0.4052
<b>CT<sub>max</sub></b>	Sex	F(1,54)= 550	< <b>0.0001</b>
	District	F(2,54)= 13	< <b>0.0001</b>
	District x Sex	F(2,54)= 3	=0.0747

#### 4.2.2 Critical thermal limits for larvae

The CT<sub>min</sub> for the larvae was marginally significant ( $F_{(2,27)}=3.4$ ;  $p=0.0465$ ; Fig. 3A) for the sampled districts. Bobirwa had a slightly higher CT<sub>min</sub> with Okavango having the lowest CT<sub>min</sub> (Fig. 3A). Critical thermal maxima was marginally significant ( $F_{(2,27)}=6.6$ ;  $p=0.0457$ ; Fig. 3B) for the districts sampled. Okavango had the highest CT<sub>max</sub> with Bobirwa reported the lowest (Fig. 3B). However, there was no significant difference between Okavango and Ngamiland for CT<sub>max</sub> (Fig. 3B).

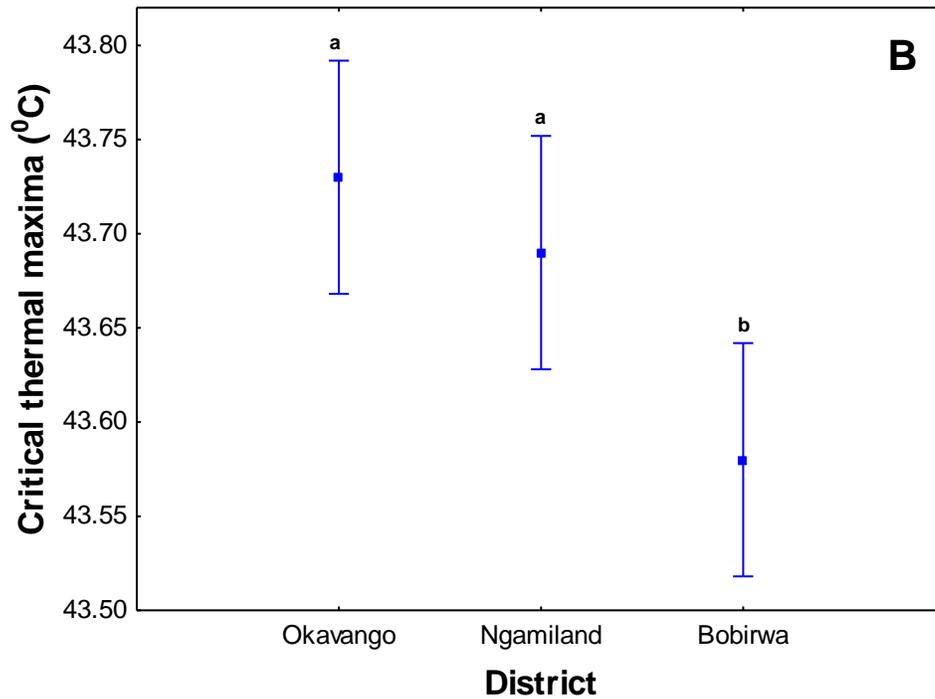
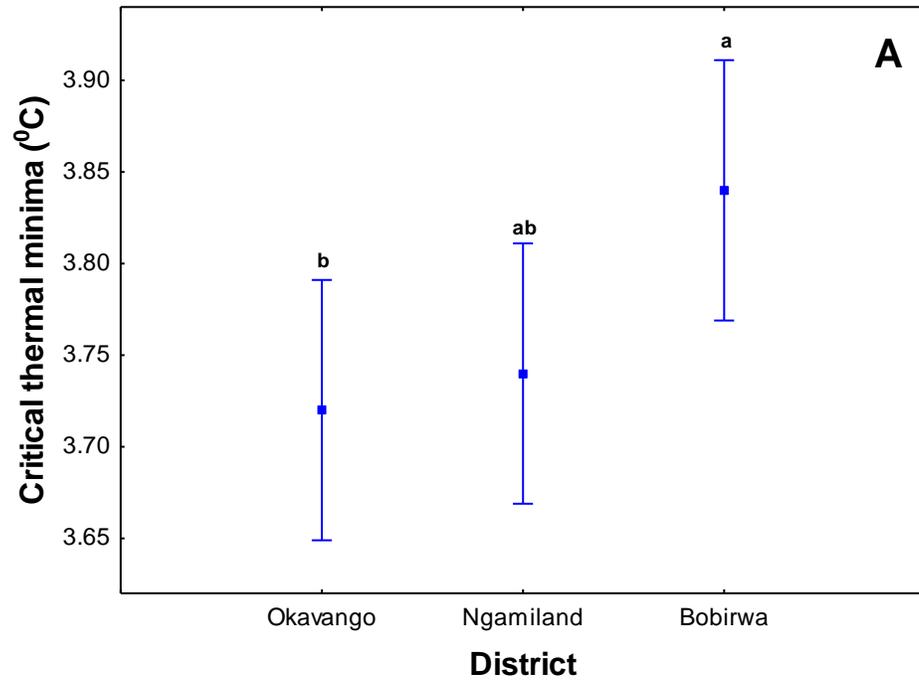


Figure 3: Critical thermal minima (A) and critical thermal maxima (B) for the *An. arabiensis* mosquito larvae. Each point represents mean  $\pm$ 95% CL. Means with the same letter are not statistically different.

### 4.3 Discussion

It is critical to establish controlled laboratory baseline thermal functional activity windows under that would provide the ecological relevance to naturally fluctuating temperatures on insect fitness (Paaijimans *et al.*, 2013). Insect species fitness is determined not only by temperature fluctuations but through several biotic (predators, parasites, parasitoids) and abiotic (humidity, rainfall, habitats) stressors (Hamby *et al.*, 2016; Hong *et al.*, 2016; Wang *et al.*, 2016; Dale and Frank, 2017). Furthermore, fitness attests for the overall organismal development, reproduction and survival (Kingsolver and Huey, 2008); Orr, 2009). The extreme environmental temperatures and the CTLs may therefore be important for understanding species distribution limits and responses to climate change (Ribeiro *et al.*, 2012; Camacho *et al.*, 2015). Climate change has brought with it, seldom increased floods (Arnell and Gosling, 2016), and thus providing ‘transient’ habitats for mosquitoes. Thus functional thermal limits measured here, will help explain the role of these transient environments in sustaining vector activity and indeed populations, with obvious implications on disease dynamics. However, the CTLs may highly be impacted not only by the methodology but start temperature, ramping rate and experimental duration (Terblanche *et al.*, 2007), body mass and ontogeny (Bowler and Terblanche, 2008; Nyamukondiwa *et al.*, 2009; Klockmann *et al.*, 2017).

The results showed mosquito females had wider thermal ranges (difference between  $CT_{\min}$  and  $CT_{\max}$ ) compared to the males, indicating differential functional activity windows that favors females (higher thermal windows) relative to the males. This is in agreement with previous work on *Anopheles* species that demonstrated more temperature plasticity in females than males (Lyons *et al.*, 2013). The reasons for the higher thermal breaths for the females relative to males are largely unknown. However, due to feeding preferences, female mosquitoes explore several habitats in search of hosts, thus may, in the process, adapt to diverse microclimates in during foraging than males, whose migration to diverse habitats is constrained (Moczek 2010; Burkett-Cadena *et al.*, 2013). This result may have a bearing in establishment of invasive mosquito vector species with implication on parasite and disease (such as malaria) establishment in new environments. Amongst other districts, Okavango had the widest CTLs range (43.73 °C to 3.73 °C) which was in agreement with widest average monthly temperature ranges (31.9 °C to 14.5 °C; Fig. 1A and B) as suggested by Pincebourde and Casas (2015) that generally insects from a higher range (max- min), have wider thermal window. This may therefore confer a long term mosquito plasticity and adaptation to climate change that in turn, may lead to high *Anopheles* mosquito- parasite abundance and diversity in the district favored by moderate annual rainfall (Table

1; Fig. 1C). Extreme temperatures in malaria transmission may not only be limited to impacting vector densities and/or adaptation (Beck-Johnson *et al.*, 2013) but may also impact parasite survival within the vector (Blanford *et al.*, 2013). Temperatures that are low (below 16 °C) arrest parasite development whilst temperatures above 30 °C have a negative effect on both development and transmission (Murdock *et al.*, 2014). The most critical feature of *Plasmodium* life-history is the extrinsic incubation period (EIP) as a temperature dependent developmental period (generally 8-14 days) inside the mosquito before the parasite becomes transmissible (Charlwood *et al.*, 1997). The EIP may be interrupted by death of adult *Anopheles* mosquitoes before they could potentially transmit the parasites. In this case, the parasite transmission becomes limited to the survival of the vector. As affirmed in other insect species by Nyamukondiwa *et al.* (2009), high temperatures significantly affect older sensitive insects, in like a manner, female mosquito vectors may die before the parasites migrate to salivary glands in making it infectious. Thus, this suggests that extreme temperatures that negatively impact vector responses and the parasite are likely to decrease malaria transmission with current and future climate change (Murdock *et al.*, 2016). In contrast, some reports state that, increasing temperatures influence potential distribution of vectors and further risks of diseases they associate with (Samy *et al.*, 2016). In addition, increasing temperatures are reported to proliferate pathogen within the vector thus posing risk of emerging and re-emerging diseases (Knox, 2011).

The CTLs for the larvae and adults in most cases differed with approximately 0.5 °C to 0.75 °C with larvae having a much narrower range than adults. It has been demonstrated by Asare *et al.* (2016) that hourly variations in water temperature are important for the larval stage developmental times though insensitive to sub-hourly variations. Although increased temperatures aid in larval development, extreme temperatures significantly increase larval mortality which has a direct impact on the survival of adult *Anopheles* population dynamics (Christiansen-Jucht *et al.*, 2014). Though extreme temperatures may have detrimental effects on the growth and survival of mosquitoes, some (mosquito vectors) remain a threat by coping with exposure to low or high temperatures. Some may employ behavioral mechanisms by seeking conducive environments to regulate their body water loss. In addition, females mitigate the issue of thermal stress by feeding on a warm-blooded host to elicit a protective heat shock response (Benoit *et al.*, 2011; Lahondère and Lazzari, 2012). During blood feeding, female mosquitoes respond to thermal stress by excreting drops of fluid through anus (process called prediuresis) which concentrates the erythrocytes (nutrients) contained in the blood meal thereby cooling the body by evaporation and restoring the water balance in the body (Lahondère and Lazzari,

2013). Furthermore, blood meal triggers off the synthesis of heatshock proteins in mosquitoes, providing evidence of thermal stress tolerance and ultimate protection (Lahondère and Lazzari, 2012). They have a role as molecular chaperones that preserve the function of enzymes and other critical proteins (Benoit *et al.*, 2011). Extreme cold temperatures negatively affect survival of mosquitoes in that others undergo diapause or hibernation. They also have ‘winter-hardy eggs’, which remain dormant until favorable conditions prevail thus enabling hatching and producing a new population, a mechanism contributing to their evolutionary success (Diniz *et al.*, 2017).

Studies have demonstrated that thermal history highly affects insect thermal tolerance (Nyamukondiwa *et al.*, 2010). These are extrinsic environmental factors that may directly or indirectly impact insect responses and thermal tolerance such as varying daily and seasonal climatic conditions (Chown and Terblanche, 2007; Overgaard and Sørensen, 2008). Fluctuating temperatures strongly affect the water and the ambient environment (inhabited by mosquitoes) with more variations in the ambient since water exhibits peculiar heat capacity (Oliver and Palumbi, 2011). It may as thus, account for the larvae to have narrower thermal window (43.73°C- 3.73°C) compared to adults (44.5°C- 3.23°C) because inherently, they are adapted to narrower thermal environments cushioned by unique water properties (Bayoh and Lindsay, 2003; Danladi-Bello *et al.*, 2017).

Overall, this study demonstrates CTLs and the ecological relevance to thermal tolerance of *An. arabiensis* (under laboratory conditions) as one of the malaria vectors in Botswana (Tawe *et al.*, 2017). Though few studies have been done to examine thermal tolerance in malaria vectors elsewhere, this study serves as a baseline on implications on planning strategies for malaria parasite transmission and vector population dynamics on changing temperature patterns. Although current perceptions of malaria control and prevention in this era are important (Tong *et al.*, 2017), it is indeed that, future malaria vector populations and parasite transmissions are based on their responses to climate change (Ngarakana-Gwasira *et al.*, 2016; Beck-Johnson *et al.*, 2017). Also, there is a need to look into differential responses across space, indicative of local adaptation based on the thermal history.

#### **4.4 Conclusion**

Female *An. arabiensis* vectors (wide thermal range) are likely to cope better with climate change compared to the males (narrow range) thus giving vectors and parasites opportunity to thrive with Okavango at more risk than Ngamiland and Bobirwa. However, temperature remains important to

the vector and parasite longevity (Blanford *et al.*, 2013; Ewing *et al.*, 2016). With this study, there is a need for further future assessment on temperature responses for other potential mosquito vectors, population age structure and effect on acclimation thus, giving an overall understanding of short and long term ecologically relevant adaptation to climate change in the country. Furthermore, there is need to incorporate the effects of temperature on other life history traits, before solid conclusions can be drawn on the effects of temperature on vector fitness traits. Nevertheless, current results remain significant in providing critical information for designing models for future vector activity and malaria transmission dynamics on local scale.

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**CHAPTER 5**  
**GENERAL DISCUSSION**

## 5.0 General discussion

Mosquito vectors for malaria and other human and animal diseases have increasingly become a looming threat for humanity (Cholewinski *et al.*, 2015; Gasperi *et al.*, 2012). Their prevalence and distribution may be deemed risky for harboring pathogens with potential transmission of diseases (Goettel, 1987; Dennison *et al.*, 2014) for both humans (Guedes *et al.*, 2017) and wildlife (Ricklefs *et al.*, 2016). In Africa, the distribution and abundance of vectors associated with malaria parasite transmission are mainly the sibling species of *Anopheles gambiae* complex and *Anopheles funestus* group (Wiebe *et al.*, 2017). Though their abundance may be a threatening factor, there are some biotic and abiotic factors that facilitate vector- pathogen interaction for a successful disease infection to happen (McMenamin *et al.*, 2016). In Botswana, *An. gambiae* sensu lato and *An. arabiensis* are considered the main malaria vectors (Simon *et al.*, 2013; Tawe *et al.*, 2017). Therefore their population dynamics and interaction with humans and the environment remains a critical area of exploration in time and space for better understanding of malaria transmission and implementing vector and associated disease control intervention strategies.

The uniqueness of vegetation cover and water bodies in malaria endemic districts of the country may be considered a supporting contributing factor in shaping mosquito vector abundance and capacity to proliferate malaria (Ricotta *et al.*, 2014; Crocker *et al.*, 2017). As it has been established in this study that large water bodies support mosquito abundance, it may be essential to consider the uniqueness of several large water bodies (river) and their capacity to sustain breeding zones for vectors in malaria endemic districts thus assisting in determining which intervention strategies may be implemented with regard to densities and the level of risk to the communities. For example, according to Simon *et al.* (2013), the country is aimed at malaria elimination, therefore strengthening vector control in districts with perennial and permanent water basins and rich floral diversity (Okavango, Chobe and/or Ngamiland) should be a priority since *An. arabiensis* larval habitats are considered to be highly productive along stream beds associated with human activities (Shililu *et al.*, 2007). Moreover, human settlements associated with large permanent water bodies may opt for integrated vector management approach in minimizing disease transmission. Personal protection (eg. Clothing, bednets) (Killeen *et al.*, 2017) and housing structures that minimize mosquito entry (Von Seidlein *et al.*, 2017a), may be obvious eminent physical barrier methods towards effective reduction of mosquito bites and high indoor resting densities exacerbated by water bodies in close proximity (Pires *et al.*, 2010). My study recommends that future settlements in the country be established at least 2km away from

permanent water bodies as a way of avoiding high mosquito densities and their dispersal zone. This minimizes their risk of infectivity that may result from interaction between mosquito- carrying *Plasmodium* parasite and human hosts (Churcher *et al.*, 2017). Future studies are recommended to further explore diversities of mosquitoes belonging to different genera (*Anopheles*, *Culex*, *Mansonia*, *Aedes* and others) and species population densities in both malaria endemic and non-endemic districts to map the distribution and assess potential risks on vector- borne diseases countrywide. Currently, there are increasingly reported cases of malaria in non-endemic districts (see Chihanga *et al.*, 2016). This anomaly may be associated with mosquito vector-parasite establishing in new environments through selection, physiological adaptation and mechanisms of phenotypic plasticity (Grenier *et al.*, 2016). Some cases may be due to erratic, patchy and unpredictably heavy rains in the southern part of the country leading to potential breeding habitats that increase mosquito populations thus interacting with humans carrying parasites from endemic zones. With this in mind, I recommend a holistic approach that may necessitate the country to consider a robust malaria monitoring system on border entry points and between endemic and non-endemic districts (see Edwards *et al.*, 2015). This may be possible by restricting movements (or quarantine individuals with malaria parasites) as in the case of virulent or life threatening emerging and reemerging diseases (Cohen, 2016). Furthermore, Okavango district as a malaria prone area and a tourist destination receiving high influx of individuals may be a priority for this initiative by screening humans for the *Plasmodium* parasite during transmission seasons. This may be established across the districts for effective and collaborative efforts towards malaria elimination.

Though rainfall may be an essential contributing factor (Sang *et al.*, 2017), humidity and temperature are confounding factors determining mosquito population abundance as affirmed by Beck-Johnson *et al.* (2013). Therefore, temperature and humidity predictive models may be relevant for the country in future mosquito population abundance and risk of malaria (see Abiodun *et al.*, 2016). Temperature plays a pivotal role not only for adult *Anopheles* vector mosquito but also for the *Plasmodium* parasite development (Noden *et al.*, 1995; Lingala, 2017). One of the robust parasites associated with most malaria cases in the country and the most countries in sub- Saharan Africa is *P. falciparum* (Gething *et al.*, 2016). This parasite demonstrated to have diverse intrinsic variations in adaptability to culture conditions *in vitro* (White *et al.*, 2016). *Plasmodium falciparum* encodes heat shock protein 70s (Hsp70) that resist thermal pressure during host–parasite interactions (eg. fever) and may export Hsp70 to the host cell for cell modification in favor of the functional adaptation for the

parasitic life style (Przyborski *et al.*, 2015). Thus, models on mosquito- parasite temperature interactions, may better explain current malaria transmission, future cases and intervention strategies in endemic and non-endemic districts (Shapiro *et al.*, 2017). This study demonstrated the potential of female *An. arabiensis* to cope with extreme temperatures since it has a wide thermal window to activity. Thus, with the current and imminent climate change, female *Anopheles* mosquitoes may adapt to extreme temperatures with the parasite hence posing risk of malaria burden in the country. Though male *An. arabiensis* mosquitoes showed a narrower thermal window and generally reported to have a shorter life span compared to females (Ndoen *et al.*, 2012), they may invest in successful deposition of their sperms once in female spermatheca such that fertilization is possibly with females for reproduction thus, enhancing population abundance (Klowden, 2006). Therefore, further assessment of microclimates on fine scale and large scale in the country may be necessary to correlate their effect on vector abundance and responses to temperature fluctuations.

With the above in mind, vector mosquito abundance has been a challenge for malaria programme worldwide especially during the transmission season (Yapabandara and Curtis, 2004). Therefore, the use of insecticides became appreciated worldwide in reducing mosquito populations as an efficacious short term way of managing vectors and their associated diseases (Raghavendra and Subbarao, 2002). In Botswana, adult mosquitoes have been controlled by insecticides for >60 years (WHO, 2012). Though insecticides brought benefits for this country over the years, my study demonstrated reduced efficacy of insecticides (especially the pyrethroids) to locally collected wild populations of female *An. arabiensis*. Therefore, the country may need to embark on programmes that monitor resistance in vector mosquito species with careful consideration of the use of persistent insecticides that may negatively impact the environment. Insecticides normally have potential to affect non-target organism which may serve as pollinators, parasitoids and food for other organisms (see Geiger *et al.*, 2010). In areas such as the Okavango where flora and fauna may be regarded as source of income through tourism, continuous use of persistent insecticides may not be recommended. Fairbrother *et al.* (2016) affirms that species may be endangered by the use of insecticides thus hampering the future biodiversity and subsequently impacting on the economy of the country. It has been found out that pesticides may acutely or chronically accumulate in organismal tissues which may potentially impact other feeders across the trophic levels (Brodeur *et al.*, 2017). Due to these effects on the natural environment, strategic conservation measures and pesticidal use on mosquito vector control may be relevant for the country. As such, modern technology on insect biotechnological approaches

have been used and appreciated for the control of malaria vector mosquitoes and may be reviewed and adopted by the country. These are molecular approaches that may target mosquito vector genetics for their control (eg. Von Seidlein *et al.*, 2017b). They may alter the mosquito- communication with the environment and/or hosts, mosquito- parasite replication machinery and survival processes as well as behavioral adaptation, making it difficult for reproduction, survival and population establishment. With these approaches, only mosquitoes may be affected for their control with minimal impact on the physical environment. Though vector control still necessitates the use of insecticides for indoor residual spraying (IRS), further consideration on the use of alternative insecticides (or rotational use) may be essential basing on the outcomes of my study on observed resistance to pyrethroids. According to Rao and Mallikarjuna (2017) botanical pesticides (plant based) are the green weapons for sustainable pest control that may serve as alternative to synthetic pesticides in managing resistance. Since they are natural derivatives, they are considered eco-friendly ‘soft’ pesticides and hence not persistent on the environment and other organisms (Dutta, 2015). Okavango district in particular, may need alternative of botanical pesticides since the study showed prominent resistance towards the pyrethroids. Despite Okavango having resistance to pyrethroids which needs to be managed, it has prestigious biodiversity that needs to be maintained for future stronger ecological foundations as in keeping with Visconti *et al.* (2016). This may be extended to other endemic districts such as the Chobe and the Ngamiland. Bobirwa district (where resistance was not prominent) may consider rotational use of pesticides with different mode of action to reduce chances of selection pressure on mosquito evolutionary genetics. Other districts such as Boteti and Tutume may do likewise as an advanced strategic approach of managing resistance in vector species. Moreover, continuous and rigorous monitoring and evaluation of resistance in malaria vectors may be an essential step before consideration of any intervention. Though may be costly for the country with bottlenecks on research and information sharing, indeed it may be essential for possibly elimination of malaria in the future of Botswana.

Overall, malaria elimination may be possible for Botswana if she considers a holistic vector and parasite management approach *vis a vis* personal protection and housing structures, environmental management and in-depth research, vector populations and resistance management, human and animal hosts, host- vector parasite interaction and drug resistance. In achieving the above, an integrated vector management driven approach by malaria control personnel’s, policy makers and other relevant

stakeholders may consider the recommendations of my work in paving a path towards elimination of malaria.

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